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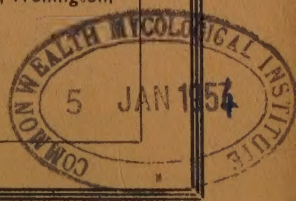

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(AGRICULTURAL RESEARCH SECTION)

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Editor: N. A. Marris, M.Sc., B. Com.
Assistant Editor: M. O'Connor, M.Sc.
Department of Scientific and Industrial Research, Wellington

VOL. 35, SEC. A OCTOBER, 1953 NUMBER 3

QUALITY AND KEEPING QUALITY OF NON-WASHED BUTTER

By F. H. McDOWALL, J. A. SINGLETON and J. J. O'DEA,
The Dairy Research Institute (N.Z.), Palmerston North

(Received for publication, 24 July 1953)

Summary

Non-washed butter has been compared with washed butter from similar cream, both under pilot plant conditions at The Dairy Research Institute (N.Z.) and under large-scale conditions at six commercial factories. The washed and non-washed butters when examined soon after manufacture were found to be of equivalent grade, even when made from cream of relatively poor quality. They showed similar losses in grade score when held at 14°F for four and eight months. The butters had similar amino-nitrogen/total-nitrogen values and butterfats extracted from the stored butters gave similar Lea peroxide tests, Schibsted fat aldehyde values, and fat acidity values. The bacterial counts were not significantly different at any stage. The results, taken in conjunction with results of similar studies in Denmark, Australia and Canada, indicate that when the cream is of reasonable quality omission of the chill-water wash is not likely to affect the quality of the butter, provided that the churnability of the cream is such that the churning temperature can be low enough to permit an adequate control of butter consistency. The increase in percentage curd content of the butter due to non-washing was within the range 0.31 to 0.64.

INTRODUCTION

Washing of the butter granules with chill-water before working has for long been accepted as a necessary stage in the making of a good quality butter. Recently, however, evidence has been brought forward to show that non-washed granules yield a high-grade butter of good keeping quality. This evidence is drawn both from experimental comparisons and from commercial buttermaking experience in a number of countries, e.g., in Denmark[1], Australia[2, 3, 4, 5, 6], and Canada[7]. Because of the importance to New Zealand of maintaining a high standard of quality in export butter (which must withstand a considerable period of frozen storage), a major innovation such as the omission of washing must be rigorously checked before it is adopted commercially. The present report supplies a brief survey of the information available from overseas and the detailed results of New Zealand investigations during 1944–1950.

EFFECT OF CHILL-WATER WASH IN BUTTERMAKING PRACTICE

1. *Hardening of the butter granules*

The wash with cold water hardens the butter granules and so facilitates: (a) the working of the butter, especially of butter which is liable to become sticky during working; and (b) packing of the butter in mechanical packing-machines immediately after removal from the churn. When the chill-water wash is omitted the buttermaker must depend mainly on alteration of cream-cooling conditions or of churning temperature for control of the firmness of the butter at the working and packing stages.

2. *Effect on curd content*

The droplets of cream serum enclosed in the butter granules as they form cannot be removed by washing. Butter from cream churned to large granules before washing retains more buttermilk than butter from cream churned to small granules. (O'Shea and Lyons[8], for example, found average curd contents of 0.493, 0.659 and 0.921 per cent in butters from small, medium, and large granules, respectively.) In addition, some buttermilk is held in the interstices between the granules, the amount so held depending on the time allowed for draining after the main volume of buttermilk has been run off. The extent of removal of this superficially held buttermilk varies with the conditions of working, as shown in Table I. Curd content of butter varies also with variation in the non-fatty solids content of the cream churned. Cream diluted with water yields butter with a relatively lower curd content, and cream concentrated by vacuum-pasteurizer treatment after preheating yields butter with a comparatively high curd content.

TABLE I. PERCENTAGE CURD CONTENTS OF BUTTER GRANULES WASHED UNDER DIFFERENT CONDITIONS (after O'Shea and Lyons[8])

Size of Granule	Badly drained	Washed once	Washed twice	Break water added and washed once
Small	0.760	0.584	0.470	0.282
Medium	0.952	0.860	0.768	0.488
Large	1.242	1.176	1.128	0.616

Since all the buttermilk solids cannot be removed from the butter granules by washing*, omission of the washing causes only an alteration in the proportion of curd substances present—it does not change the character of all the aqueous droplets. Both washed and non-washed butters contain sufficient lactose and protein to support bacterial growth when the conditions for such growth are favourable.

* From lactose estimations McDowall and McDowell[9] estimated that washed butter contains about 10 per cent of buttermilk.

The maximum curd content of butter from cream with a serum containing 9 per cent of non-fatty solids is 1.5 to 1.6 per cent, calculated on the assumptions that:

(a) all the water present in the butter is derived from the cream serum;

(b) there is no mechanical entrainment of curd particles during granule formation; and

(c) there is no adsorption of milk solids on to the fat globules enclosed in the butter granules,

Example:—

Assume moisture content	= 15.9 per cent
Non-fatty solids in 15.9 parts of water	$= \frac{9}{91} \times 15.9$
	= 1.58 parts
Hence curd content of butter	= 1.58 per cent

Factor (a) will apply even for non-washed butter only when no make-up water is required or when the make-up moisture is added in the form of buttermilk (see discussion below); factor (b) does not apply for sour cream butter, because with sour cream some curd particles are caught up by the granules during churning; and factor (c) may have some influence on the curd content of sweet cream butter. The normal curd content of New Zealand washed butter is 0.7–1.0 per cent. The expected rise with change to non-washing would be 0.5 (approx.), giving a curd content of 1.2–1.5 per cent in the non-washed butter. Butters made by the Alfa and Fritz continuous processes carry the full load of non-fatty milk solids corresponding to the final moisture content. They thus are of equivalent composition to non-washed churned butter.

3. *Size of Moisture Droplets in Butter*

The moisture droplets incorporated into the butter during working are larger than those already present in the granules. They consist mainly of the liquid left between the granules at the final draining, which in washed butter will be chill-water containing a small proportion of buttermilk and in non-washed butter will be mainly buttermilk. Since bacteria grow mainly in the large aqueous globules, adoption of non-washing conceivably could increase the danger of bacterial deterioration of the butter, especially if the make-up water is added in the form of buttermilk. The Danish workers[1] reported that in the making of non-washed butter for storage the butter should be firm, with the aqueous portion in small droplets.

4. *Quality of Water used as Wash Water*

Washing of the butter introduces a danger of contamination of the butter with spoilage organisms, especially organisms of the *Pseudomonas* group, which may be present in water supplies accepted as suitable for domestic purposes. While contamination of the butter can be prevented by chlorination of the water supply, elimination of the washing stage is likely to be a more reliable safeguard at factories where the water supply is of questionable quality, provided that the omission has no serious disadvantageous effect on butter quality.

5. *Effect on Economics of Buttermaking*

(a) Provision of chill-water for butter washing is an appreciable proportion of the total refrigeration costs for butter manufacture, and the washing procedure takes up some time and therefore involves some labour costs. But if in the making of non-washed butter lowering of the churning temperature is found necessary in order to control the consistency of the butter, the resultant longer churning time would partly offset the gains in refrigeration and labour costs. Newman[2], on the basis of four years' experience in the manufacture of non-washed butter, reported that there was a saving of 20 minutes for each churning of butter made; while Nichols[6] found that the time saving through elimination of the chill-water wash was almost exactly balanced by the increased churning time owing to the lower temperature of churning which he found necessary. In this respect conclusions may be different according to whether the butter after manufacture is to be packed by hand or by machine.

(b) In New Zealand, where the legal maximum moisture content is 16.0 per cent and the legal maximum salt content for export butter is 2.0 per cent, non-washing increases the yield by increasing the curd content of the butter (see above, and also later), and by slightly increasing the average moisture and salt contents per unit weight of fat present. The curd content of butter is not of any economic importance in countries where the only legal limitation covering butter composition is the requirement that the fat content shall not be below 80.0 per cent, since extra moisture can be introduced to control the fat content as desired.

OVERSEAS RESULTS ON NON-WASHING OF BUTTER

Danish workers[1] reported that non-washed butter made in metal churns was not inferior in keeping quality to washed butter. Jensen *et al.*[3] in Australia found that omission of the washing stage in the manufacture of Victorian butter raised the curd content from 0.9 to 1.5 per cent without affecting the keeping quality. Pont[4] examined butters from 74 pairs of churnings from similar creams in five Victorian factories selected to give a range of cream type and grade. The make-up water for moisture control was added as buttermilk, thus eliminating possible contamination from the wash water in the experimental churning, i.e., in the non-washed butter. When graded within one week of manufacture the butter showed a difference in only two instances, when the higher grade was awarded to the non-washed butter (1 grade point higher). After 3 months' storage the washed butter was graded higher in 36 out of 68 comparisons, the non-washed butter was graded higher in 16 comparisons, and no distinction was made in 16 comparisons. The mean loss of grade in the washed butter was 1.23 points and in the non-washed butter was 1.71 points, a statistically significant difference. Pont did not find any relationship between inferior quality in the non-washed butter and difference in curd content, initial quality or results of bacteriological examinations. The average curd content of the washed butter was 1.10 per cent and of the non-washed butter was 1.52 per cent, i.e., an increase of 0.42 through non-washing. Pont concluded that the mere presence of a larger amount of curd, within the range quoted, was not a determining factor to the keeping quality. In a later investigation[5] he correlated curd content of non-washed

TABLE II. AVERAGE CURD CONTENTS OF WASHED AND NON-WASHED BUTTERS AT FIVE VICTORIAN FACTORIES (after Pont[4, 5])

Butter	FACTORY					
	A	B	C	D	E	A–E
Washed	1.00	0.95	1.24	0.90	0.88	0.99
Non-washed	1.38	1.54	1.55	1.37	1.20	1.43
Difference	0.38	0.59	0.31	0.47	0.32	0.44

TABLE III. COMPARISONS OF GRADING RESULTS ON WASHED AND NON-WASHED BUTTERS FROM FIVE VICTORIAN FACTORIES (after Pont[5])

Description of Butter	Factory	Number of Comparisons	GRADING		
			Washed butter (W)	Non-washed butter (NW)	Change in Grade due to Non-washing (NW–W)
Fresh	A	31	92.37	92.22	—0.15
	B	30	92.23	92.03	—0.20
	C	15	92.93	92.87	—0.06
	D	16	92.75	92.75	±0.
	E	10	93.00	93.00	±0.
Held for 2–3 weeks at room temperature	A	31	91.09	91.06	—0.03
	B	30	90.73	90.57	—0.16
	C	15	90.77	90.77	±0.
	D	16	90.51	90.56	±0.
	E	10	92.60	92.30	—0.30
Held for 4–5 months at 16° F.	A	27	90.70	90.50	—0.20
	B	30	90.47	90.17	—0.30
	C	15	89.87	89.87	±0.
	D	16	90.38	90.25	—0.13
	E	—	—	—	—

butter with quality of the butter after holding at 60–70° F. for two weeks, or at 50–60° F. for three weeks, and at 16° F. for 4–5 months.

Make-up water, as in the previous trial, was added as buttermilk. The non-washed and the control butters were made in the same churn, and the chill-water used for the washing in the control churning was of good quality and was chlorinated. The butters were made from creams of moderate acidity (0.30–0.45 per cent lactic acid), except at one factory, where they were made from sweet cream. The average curd contents are shown in Table II, and the grading results are given in Table III.

The rise in percentage curd content resulting from non-washing varied from 0.31 to 0.59. The differences in grade were small, but any differences were in favour of the washed butters. On the whole, however, there was no evidence of any appreciable difference in keeping quality or in bacterial count between the washed and the non-washed butters (see also Table IV). There was a somewhat higher yeast count in the non-washed butter.

TABLE IV. AVERAGE CHANGES IN GRADE AND AVERAGE BACTERIAL COUNTS IN WASHED AND NON-WASHED BUTTER DURING STORAGE (after Pont[5])

		Fresh	Holding at room temperature for 2–3 weeks	Storage at 16° F. for 4–5 months
Average Change in Grade	Washed	—	1.54	2.03
	Non-washed	—	1.50	2.11
Average Bacteria Count	Washed	61,000	93,000	—
	Non-washed	80,000	126,000	—

The non-washed butter had higher copper and iron content, as could be expected from the higher curd content, since heavy metals are attached to the protein in the serum; but this additional metallic contamination did not increase the extent of oxidation of the fat, probably because the additional antioxidant effect provided by the extra curd present countered the pro-oxidant effect of the increased copper content. When sweet cream was used there was no appreciable difference between the oxidation tests for the butterfats from the washed and the non-washed butters.

Nichols[6], from a somewhat similar study, in Queensland, concluded that it is possible to manufacture good quality non-washed butter, but that a churning temperature at least 4°F. lower than normal was necessary, to compensate for the loss of the hardening effect of the wash water, i.e., to produce a butter which would withstand thorough working. The increase in the percentage curd content due to non-washing was 0.44. The keeping quality of well-made non-washed butter during 4 months at cold storage temperatures was closely comparable with that for washed butter; but when the non-washed butters were badly made there was evidence of the effect of the poorer texture on the keeping quality. There was no significant difference in the keeping quality of pats cut by hand from the washed and non-washed butters and held at 40–50° F. for 4 weeks, provided that the butter was worked

well enough to prevent bacterial deterioration. According to Nichols the higher curd content of the non-washed butter had a slight anti-oxidant effect, causing the fat to have a lower peroxide value. When the butter was reworked before patting, however, there was a development of condensed-milk flavour in some of the non-washed samples, resulting in a 2–5 point drop in grade. Nichols concluded that non-washing is not advisable for butter that is to be vacuum-worked before patting.

Arnott, Green and Gibson[7] compared the grade scores of 239 churnings of commercially manufactured washed butter with those of 337 churnings of non-washed butter over a 3-month period. They found a greater percentage of "40 score" (high-scoring) butter among the non-washed butters. They found, too, that batches of washed and non-washed butter made from the same lot of cream showed similar flavour scores after 45 days' storage at –20° F. The average curd contents of the washed and non-washed butters were 0.8 and 1.5 per cent respectively. Bacteriologically there was no essential difference between washed and non-washed butter samples, and the higher curd content of the non-washed butter did not stimulate bacterial growth significantly.

Experimental Results

These were obtained partly on butters made at The Dairy Research Institute (N.Z.) experimental factory and partly on butters from comparative churnings in commercial factories.

Institute results

The butters were made in churnings of 150–200 lb. of butter in a small internal-worker churn, from cream treated in a Vacreator vacuum-pasteurizer. Several series of comparisons were made on butters from good quality creams (see Table V), and a single but more extensive series of comparisons was made on butters from poor quality creams (see Table VI). The poor quality creams were obtained by holding factory-separated cream at summer room temperatures for two days prior to neutralization and pasteurisation in a vacuum-pasteuriser. At time of treatment they were classed as poor quality first grade or (mainly) second grade, and had acidities ranging from 0.20 to 0.60 per cent lactic acid. They were, therefore, of poorer quality than the cream normally received at New Zealand butter factories.

Commercial Factory Results

Arrangements were made with six commercial factories to supply, during February, 1947, butters from a pair of comparative churnings, one washed and the other non-washed, from the same vat of cream, on three successive days. The water used for washing and for make-up purposes was the normal chill-water supply for each particular factory. Three 56-lb. blocks of butter were set aside from each churning. They were packed in boxes carrying the Institute brand and were marked with code letters, so that all comparative gradings were made "blind". One box of the three from each churning was graded "fresh" according to the normal commercial routine, and the remaining two boxes were

TABLE V. GRADING RESULTS FOR SEVERAL SERIES OF COMPARISONS OF WASHED AND NON-WASHED BUTTERS MADE AT THE DAIRY RESEARCH INSTITUTE (N.Z.) FROM GOOD QUALITY CREAM

(Control butters graded 93—94 points)

Series	Churning No.	Treatment	No. of Comparisons	Change in Grade due to Non-washing* (A and B: 2 minus 1; and C and D: 2 minus 1; 3 minus 1)				
				Fresh Butter			4 months	8 months
				Panel A	Panel B	Panel C	Panel B	Panel B
A	1.	Washed	5					
	2.	Non-washed (make-up water as buttermilk)		—0.25	—0.05	+0.26	+0.45	+0.13
B	1.	Washed with chill-water	4					
	2.	Washed with chilled buttermilk		+0.06	—0.01	±0.	+0.22	+0.26
C	1.	Washed	8					
	2.	Non-washed (no make-up water)		+0.09	—0.77	—0.29	—0.50	—0.50
	3.	Non-washed (make-up water as buttermilk)		+0.37	—0.48	—0.39	—0.28	—0.47
D	1.	Washed	3					
	2.	Washed with chilled buttermilk		+0.08	—0.17	—0.10	—0.25	—0.42
	3.	Non-washed (make-up water as buttermilk)		±0.	—0.50	—0.20	—0.25	—0.42

* + signifies rise in grade, i.e. non-washed butter of higher grade.
— signifies fall in grade.

placed in storage at 14° F. for 4 and 8 months respectively. The butters after storage were arranged in pairs in random order for each factory, and they were examined independently by a panel of ten persons most of whom were professional butter graders. The results, averaged for the three churnings for each factory, are given in Table VII.

TABLE VI. COMPARISON OF GRADINGS OF WASHED AND NON-WASHED BUTTERS MADE FROM POOR QUALITY CREAM AT THE DAIRY RESEARCH INSTITUTE (N.Z.) (Series E, 28 comparisons)

Age of Butter at time of examination	Graded by	(1) Normal Churning	(2) Non-washed; Churning at normal Temperatures		(3) Non-washed; Churning at lower Temperatures	
		Average Grade	Average Grade	Change in Grade due to Non-washing	Average Grade	Change in Grade due to Non-washing
Fresh	Panel A	91.43	91.26	—0.17	91.17	—0.26
	Panel B	91.56	91.33	—0.23	91.14	—0.42
	Panel C	91.08	90.94	—0.14	91.13	+0.05
	Averages	91.36	91.18	—0.18	91.15	—0.21
4 Months	Panel B	90.67	91.0	+0.33	90.66	—0.01
8 Months	Panel B	90.06	89.93	—0.13	89.98	—0.08

TABLE VII. AVERAGED GRADE SCORE FOR THREE CHURNINGS OF WASHED AND NON-WASHED BUTTERS MADE AT EACH OF SIX COMMERCIAL FACTORIES (W = Washed; NW = Non-washed)

Factory	Grade Scores of Butters when Graded at Age indicated								
	Fresh			4 Months			8 Months		
	W	NW	NW–W*	W	NW	NW–W*	W	NW	NW–W*
A	94.00	94.25	+0.25	93.00	93.10	+0.10	91.93	92.00	+0.07
B	93.50	93.5	±0	92.93	92.58	—0.35	92.03	92.00	+0.03
C	93.50	93.67	+0.17	93.03	93.18	+0.15	92.20	92.20	+0
D	94.00	94.00	±0	93.05	93.03	—0.02	92.60	92.37	—0.23
E	94.00	94.00	+0	92.80	92.86	+0.06	92.57	92.47	—0.10
F	94.00	94.00	±0	92.11	92.61	+0.50	91.73	92.03	+0.30
A–F	93.83	93.90	+0.07	92.82	92.89	+0.07	92.18	92.18	±0

*Figure in this column represents change in grade due to non-washing.

Discussion of Results

Quality of Fresh Butter

There were only slight differences between the average grades of the washed and the non-washed butters. In some of the Institute series of comparisons with good quality cream (Table V), the non-washed butter was considered by one or other of the grading panels to be of slightly better quality, but the preference more frequently was for the washed butter. In most of the series of comparisons, however, the differences were so small as to be of little commercial significance. This applied even for the butters from poor quality cream (Table VI), in which the larger quantity of residual off-flavoured serum retained in the non-washed butter could be expected to accentuate the off-flavours noted in all the butters in this series. In the comparison on factory-made butter (Table VII) the difference in grade between washed and non-washed butter in the fresh state was not commercially significant (the small differences reported for butters from two of the factories were in favour of the non-washed butter).

There were no adverse comments on the physical properties of the non-washed butter except that from Churning 3 in Series E, Table VI, the butters were found occasionally to be brittle in body, apparently because of the lower churning temperature.

The results in Tables V–VII, taken in conjunction with those reported by overseas workers and cited in detail above, would seem to establish that omisson of the wash with chill-water does not cause any appreciable loss in grade of butter examined within a few days after manufacture.

Grading of Butter after Storage

In Series A and B (Table V), non-washed butter was found to be slightly preferable at both four and eight months after manufacture, whereas in Series C and D the difference was 0.25–0.50 points in grade score in favour of the washed butter. In Series E (Table VI, butters from poor quality cream), the non-washed butters from Churning 2 (normal churning temperature) were found to be preferable at four months after manufacture, but the washed butters were slightly preferred to the non-washed butters from Churning 3 at 4 months and from Churnings 2 and 3 at 8 months after manufacture. For the commercial butters (Table VII) there was no appreciable difference in grade between the washed and the non-washed butters either at four months or at eight months after manufacture.

The indications from the grading results on the stored butters, therefore, are that non-washing did not have a commercially significant effect on the keeping quality, even of butters from relatively poor quality cream. The use of buttermilk instead of water to supply the make-up moisture (Series A, C and D, Table V) did not influence the quality of either the fresh or the stored butters.

Oxidation tests

Some results are given in Table VIII. Similar results were obtained in comparisons on butterfats from the butters made in the Institute

TABLE VIII. PEROXIDE AND FAT ALDEHYDE VALUES OF BUTTERFATS FROM BUTTERS IN COMMERCIAL TRIAL (cf. Table VII) AFTER 8-MONTHS' STORAGE AT 14°F.

(Figures cited are averages for three samples)

Factory	Lea Peroxide Value		Fat Aldehyde Value	
	Washed	Non-washed	Washed	Non-washed
A	0.25	0.32	0.36	0.23
B	0.30	0.45	1.3	1.7
C	0.25	0.25	0.30	0.34
D	0.25	0.26	0.30	0.27
E	0.25	0.29	0.21	0.22
F	0.27	0.27	0.37	0.52
A–F	0.26	0.31	0.47	0.55

trials, including those from the butters made from poor quality cream (see Table VI). There were no consistent differences in the Lea peroxide or in the Schibsted fat aldehyde values of the butterfats from the washed and the non-washed butters.

Amino-Nitrogen Content and Fat Acidity Values

Non-washing did not cause any increase in the amino-nitrogen/total-nitrogen ratios in the butters, or in the fat acidity values for the butterfats from the butters, after 8 months' storage (see Table IX). Similar results were obtained with samples from the Institute trials (Tables V and VI).

TABLE IX. AMINO-NITROGEN/TOTAL-NITROGEN RATIO IN WASHED AND NON-WASHED BUTTERS FROM COMMERCIAL TRIAL (see Table VII) EXPRESSED AS A PERCENTAGE OF THE TOTAL NITROGEN PRESENT

Factory	% Nitrogen as Amino-nitrogen		Fat Acidity (as % Oleic Acid in the Butterfat)	
	Washed	Non-washed	Washed	Non-washed
A	3.6	3.9	0.18	0.18
B	4.3	4.3	0.15	0.17
C	3.8	3.8	0.20	0.19
D	3.6	4.0	0.14	0.13
E	3.4	3.6	0.17	0.17
F	3.8	3.9	0.17	0.17

Effect of Lowering of Temperature of Churning on Churning and Working Times

Wash water may be at a temperature up to 5° F. below the temperature of the cream at commencement of churning. Even when it is at the temperature of churning it has a hardening influence on the butter in the granules, because of the rise in temperature that occurs during the churning process. For Churning 3 of Series E trials (Table VI) the churning temperature was 3° F. lower than the temperature for the control churning. The average churning and working times are given in Table X. The effect of lowering of churning temperature

TABLE X. EFFECT OF 3°F. LOWERING OF CHURNING TEMPERATURE ON CHURNING AND WORKING TIMES (Series E, Table VI)

	Times (in minutes)	
	Churnings 1 and 2	Churning 3
Average time to breaking stage	36	49
Average time between draining and first moisture test	8.6	8.0
Average time to completion of working	13.0	12.6

by 3° F. on the churning time can be expected to vary considerably with type of cream and with variation in the temperature for the control churning. The difference in churning times cited in Table X, viz., 13 minutes, is the average of differences ranging from 3 to 38 minutes. These results do not necessarily represent the conditions that would apply in any one commercial factory, but their indications, so far as they go, are that if a 3°F. lowering of churning temperature is taken as desirable for the churning of cream when non-washed butter is being made, the increase in churning time is sufficient to offset much, if not all, of the saving in time from the omission of the chill-water wash—in agreement with the finding of Nichols[6] (see also above). It was found, however, that the 3°F. lowering of churning temperature in some instances caused the butter to be faulty, mainly brittle, in body. Whether or not these lower churning temperatures are necessary in the making of non-washed butter will, as stated above, depend on the type of cream being handled, the normal churning temperature adopted, and the physical condition required in the butter after removal from the churn. This condition will of course vary with the system of packing of the butter. In some New Zealand factories where non-washed butter has been made commercially over a period, it has not been found necessary to lower the churning temperature.

Effect of Non-washing on Colour of butter

The firming-up of the butter granules resulting from the chill-water wash gives the butter during working a somewhat greater resistance to compression by the rollers, and therefore provides more

effective working. It was observed during the Institute trials that some of the non-washed butters had a slightly higher colour than the washed butters, but this difference was not always apparent.

Effect of Non-washing on Curd Content of Butter

The results for the five series of trials at The Dairy Research Institute (N.Z.) and for the commercial trial are given in Table XI.

TABLE XI. CURD CONTENTS OF BUTTERS FROM WASHED AND NON-WASHED GRANULES

Series	Factory	Curd Content of Butter				
		W	NW Churning 2	Difference	NW Churning 3	Difference
		per cent	per cent	per cent	per cent	per cent
A	Dairy	0.80	1.18	0.38	—	—
B	Research	0.83	1.14	0.36	—	—
C	Institute	0.95	1.41	0.46	1.49	0.54
D		0.92	1.42	0.52	1.54	0.64
E		0.86	1.27	0.41	1.27	0.41
F	Commercial Factory					
	a	0.90	1.27	0.37	—	—
	b	0.80	1.11	0.31	—	—
	c	1.06	1.44	0.38	—	—
	d	1.03	1.36	0.33	—	—
	e	0.93	1.27	0.34	—	—
	f	0.98	1.51	0.33	—	—
	a–f	0.95	1.33	0.38	—	—

The increase in percentage curd content due to non-washing varied from 0.31 to 0.68 (compare earlier discussion on factors affecting the expected curd content rise). Under commercial factory buttermaking conditions, the average rise in curd content was 0.38 per cent, i.e. approximately the same as found by Nichols for Queensland butters[6] and somewhat below the value calculated above as the maximum expected rise with normal undiluted cream. A rise of 0.40 in percentage curd content would cause a rise of approximately 0.50 in the percentage overrun.

Conclusion

The evidence assembled above, both from overseas work and from the present series of trials in New Zealand, leads to the conclusion

that omission of the washing stage does not cause any appreciable alteration in the quality or in the keeping quality of butter from cream of fair quality that has been treated in a Vacreator for removal of off-flavours. When cream contains strong off-flavours associated with the serum, it can be accepted that removal of as large a proportion of the serum as possible will be an advantage, and that then the wash with chill-water should be retained as a necessary stage in the buttermaking process. The chill-water wash may be necessary under some circumstances also, to harden up the butter granules and permit satisfactory working of the butter or to facilitate immediate packing in mechanical packers. While non-washing has the advantage that it reduces the cost of manufacture of butter and that it increases the yield of butter (in countries where there is a 16 per cent legal maximum for moisture content), the indications are that a suitable chill-water supply should be provided for in every butter factory and be kept available for use when required. The observation of Nichols[6], that non-washed butter had a greater tendency to undergo deterioration after vacuum-reworking, has not been checked in the present investigation.

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STUDIES IN MONOZYGOTIC CATTLE TWINS

X. GENERAL SUMMARY

By John Hancock, Ruakura Animal Research Station, Department of Agriculture, Hamilton, New Zealand

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Summary

The recent literature on cattle twins is briefly outlined and discussed in relation to the results presented in the previous papers of this series.

Introduction

It is almost ten years since the first twin-sets were located on which the foregoing studies have been based. Over this period new information has accumulated confirming or refuting previous conclusions or opening new paths in twin research. This paper is an attempt to summarise the most important aspects of recent work and with direct bearing on the papers of this series. For a more detailed and extensive review of cattle twin work the reader is referred to Hancock[1].

1. Frequency of Monozygotic Twinning

On the basis of twinning statistics appearing in the literature, Johansson and Venge[2] calculated and tabulated the probable frequencies of monozygotic twins in various cattle breeds. The total number of births involved was approximately 275,000, of which 5,000 were twins. Monozygotic twinning rates in per cent of total births varied from 0.05 in Finnish Ayrshires to 0.28 in German Lowland cattle. Expressed as percentage of like-sexed twins the monozygotic twinning rate varied from 6.0 in the Simmentaler breed to 26.8 in the Swedish Polled Landrace breed. Although very wide, these differences were not significant. The authors therefore concluded that the weighted means, i.e., 0.11 per cent of all births and 10.6 per cent of all like-sexed twins, provided the best estimate of monozygotic twinning in all dairy breeds.

Data collected from the literature pertaining to the frequency of monozygotic sets of calves of higher order than twins, were presented by Johansson[3] (triplets), and Donald, Biggar and Logan[4] (quadruplets, quintuplets and sextuplets). There were more like-sexed triplet sets than would be expected from monozygotic twinning frequencies found in other populations. However, as the number of sets involved was very small (37), no definite conclusion could be drawn. The sex distribution in quadruplets and quintuplets did not suggest that monozygotic twinning was important in their production.

2. Diagnosis

General papers on diagnosis of cattle twins have been published by Nadai[5] and Johansson and Venge[2]. The latter workers confirmed Hancock's[6] conclusions regarding the relative usefulness of such

criteria as hair whorls and muzzle prints. They also attempted to assess the value of various linear body measurments as aids in diagnosis. Nadai[5] studied the blood vessel patterns in the fundus region of the eyes of many (mainly dizygotic) twins, but could not reach any definite conclusions as to the usefulness of this character. He also claimed that muzzle prints were good diagnostic aids. This was supported by work of Hirsch, Graham and Dracy[7] and Dracy, Graham and Hirsch[8], but the work of Johansson and Venge supports Hancock's view that muzzle prints are of limited value.

White[9] investigated the possibility of using various properties of the blood such as haemoglobin content, red cell fragility, red cell count, plasma chlorides, etc., as a basis of zygocity. In general, the results were either inconclusive or negative.

The work at the Wisconsin school by Ferguson[10], Ferguson, Stormont and Irwin[11], Stormont, Irwin and Owen[12], Stormont and Cumley[13] and Stormont[14] on the antigenic components in cattle red cells, created great interest amongst workers in twin research. (Bonnier and Hansson[15] and Hancock[6]). At least 38 distinct red cell factors were demonstrated which were thought to be inherited as separate entities (with presence dominant over absence). On this basis it could be computed (Irwin[16]) that the number of possible combinations was so large that it was practically impossible to find two animals except identical twins with the same red-cell combination. However, the later work by Stormont, Owen and Irwin[17] established that many of the factors were not inherited independently but rather in groups of several factors together. As a result of the analysis of a series of test matings, evidence was presented that, of the 38 blood factors, 21 were the members of one system (B), seven members of another system (C), and two members of a third system (FV). Thus, the maximum number of independent genes controlling the various antigens cannot be greater than 11. The B system consists of at least 80, and the C system of at least 22 allelic groups.

Even with only 11 independent genes, red cell antigens might well have provided a most useful criterion on zygocity, had it not been for the fact, discovered by Owen[18], that dizygotic cattle twins in the majority of cases possessed identical red cell factors. The explanation for this unexpected situation was that the vascular anastomosis which occurs in the majority of cattle twin foetuses (Lillie[19]) allows an admixture of foetal blood. Since the blood factors exchanged in this way would not persist in the host calf after the normal life span of red cells and yet were found there throughout the life of the host, it was concluded that there can be an interchange of erythrocyte precursor cells which becomes established in the haemopoietic tissue of the host twin. Here they produce red cells of an antigenic pattern determined by the genotype of the donor.

At the present time, the potential usefulness of blood typing as an adjunct in the diagnosis of zygocity in cattle twins is based on two findings (Stormont[20]):

(1) Mixture of red cells with different antigenic properties can be detected in some cases by quantitative serological tests.

(2) The factor – J – which is not a true red cell antigen but only adsorbed to the cells, may cause dizygotic twins with foetal anastomosis to differ in their reactions to J antiserum (Stormont[21]). It seems further that in some cow populations the gene controlling the J factor is of an optimum frequency, i.e., about 0.5, from the point of view of greatest possible usefulness in the diagnosis of zygocity. Thus, both of these tests can serve to eliminate a number of perhaps otherwise doubtful cases of dizygotic twins.

As a result of previous experimental evidence on the behaviour of skin grafts in mammals, it was thought (Anderson, Billingham, Lampkin and Medawar[22]) that skin transplantations between members of sets of twins might provide a very good criterion of the zygotic origin of the twins. Such skin grafts (homografts) are destroyed in genetically diverse animals by a mechanism which belongs to the general category of actively immune reactions. The initial healing is as good as in grafts from one part of the body to another of the same animal (autografts), but the survival time of homografts in unrelated cattle is only approximately ten days. The results of several exhaustive series of skin transplantations involving unrelated animals, close relatives, dizygotic and monozygotic twins were described by these authors and by Billingham, Lampkin, Medawar and Williams[23]. Conclusions were as follows:—

All cattle twins show either absolute or a high degree of tolerance to homografts. The mutual exchange of skin grafts is therefore a technique which does not allow differentiation between monozygotic and dizygotic cattle twins.

Of 42 individual cattle of either sex, i.e., members of unlike-sexed sets, that received homografts from their co-twins, 36 (or approximately 86 per cent) proved to be completely tolerant within the limits of observation. The close correspondence of frequency of homograft-tolerant unlike-sexed twins with the frequency of "freemartins" justified the presumption that the tolerance to homografts exchanged between dizygotic twins is also a consequence of anastomosis of the foetal circulation. Medawar[24] showed that there was some degree of overlap between the antigens of leucocytes and of skin epithelium. Thus, it seems that foetal anastomosis in cattle twins allows not only the exchange of hormones and red cell precursors, but also of leucocyte precursors.

There was some evidence that, when skin was transplanted from an unrelated test animal to each member of a dizygotic twin set, the twins reacted with slightly different intensity. However, when grafts were transplanted from a dam to each of her dizygotic twins, these reacted in markedly different ways. Why twins that are tolerant to grafts from each other should be intolerant to grafts from their mother was not clear, but this

asymmetry of response provides a promising basis for the development of a reliable method of identifying dizygotic cattle twins.

In conclusion, it must be admitted that, despite the fact that more than two decades have passed since Kronacher began his studies on cattle twins, no single test or series of tests yet exist by which it is possible to distinguish between monozygotic and dizygotic cattle twins in an objective manner. This, however, does not imply that the diagnosis of zygocity in cattle twins is more difficult or less precise than in human twins. Newman, Freeman and Holzinger[25] showed clearly that in man a trained observer, after only a cursory examination, was able to distinguish between monozygotic and dizygotic twins with a very great degree of certainty. A similar situation applies to cattle. An observer with highly developed critical faculties can easily note a great number of often subtle characters which make each individual different from all others. As this applies even to the members of monozygotic twin sets, the real difficulty lies in knowing what kind and degree of differences are compatible with monozygocity. Full assurance in regard to this problem can only come from further systematic work.

3. Efficiency

The greater efficiency of monozygotic twins over ordinary animals depends on the fact that in a twin experiment it is possible to disentangle the hereditary variations amongst the experimental animals from the error variance. The consequent reduction of "error" in the analysis of the results increases the statistical efficiency or sensitivity of treatment comparisons.

Bonnier, Hansson and Düring[26] first attempted to define a method whereby the relative efficiency of monozygotic twins could be estimated. They thus computed "E" (the number of ordinary animals in each of two groups that one set of monozygotic twins can replace without loss of statistical precision) as the ratio

$$(1) \qquad \frac{M_B}{M_W}$$

where M_B is the mean square between sets of twins and M_W the mean square within sets of twins derived from a trial in which a number of twin sets have been treated in as nearly uniform a manner as practicable. Dick and Whittle[27], however, pointed out that, instead of using the mean squares (variances), the respective variance components should be used in the ratio. Translated into mean squares, the formula they suggested for "E" became

$$(2) \qquad \tfrac{1}{2}\left\{\frac{M_B}{M_W} - 1\right\}$$

In comparing the results obtainable by these formulae, it may be noted that the first formula gives values approximately twice as high as the second. As the use of the latter formula may provide

twin efficiency values smaller than unity, Carter[28] finally suggested that "E" should be estimated as

$$(3) \qquad \frac{1}{2}\left\{\frac{M_B}{M_W} + 1\right\}$$

Dick and Whittle[27] indicated that it was possible to estimate twin efficiency values not only from uniformity trials, but also from data accumulated in experiments involving sets of twins with the members subjected to different treatments. It may be noted that twin efficiency values derived from uniformity trial data are valid only if heredity x environment interactions are unimportant, while an efficiency value derived from a split-twin experiment presumably indicates accurately the relative efficiency gained by using twins in the particular experiment from which the value was derived.

Bonnier, Hansson and Düring[26] deduced that the efficiency of monozygotic twins relative to ordinary animals decreases in trials which involve more than two treatments, and also estimated, on theoretical grounds, the extent to which the efficiency was reduced. However, in this they did not, as shown by Dick and Whittle[27], take into account the fact that the most suitable experimental design in which twins may be used (incomplete randomized blocks) allows, in addition to direct, also indirect, treatment comparisons. In neglecting the indirect comparisons, the previous authors seriously under-estimated the relative efficiency of monozygotic twins in trials involving several treatments. The value can be computed correctly, as suggested by Dick and Whittle[27], with the aid of the general formula

$$\frac{nE}{2(n-1)}$$

where $\begin{cases} n = \text{the number of treatments} \\ E = \text{the efficiency value for twins when used in a two-treatment trial.} \end{cases}$

These authors also considered fully various statistical designs suitable for trials involving twins as experimental material.

Table I contains a summary of the twin efficiency values obtained at Ruakura (Hancock[29, 30, 31, 32] and James[33]). The twin efficiency values given in Hancock's papers would all be one unit higher if computed according to Carter's[28] method. Earlier attempts to estimate twin efficiency values were reported by Bonnier, Hansson and Düring[26], who found E = 50 for growth between 30 and 180 days and E = 5 for total calorific output of milk and growth. The latter figure was, however, derived from a split twin experiment. White[9] concluded from a trial involving four monozygotic and four dizygotic twin-sets, that monozygotic twins had no advantage in experiments involving plasma chlorides, fragility and red cell counts. Olson[34] reported, without giving numerical data, a uniformity trial on various semen characters in monozygotic bull twins and triplets. Finally, from growth trials involving 21 sets of monozygotic twins, Thoele and Hervey[35] computed twin efficiency values of 6–24 for body weight and 14–43 for height at withers.

TABLE I. TWIN EFFICIENCY VALUES

Reference	Characteristic	Twin Efficiency Value	No. of sets used
Hancock[29]	Milk	22	7
	Butterfat	54	7
	Casein	50	7
	Butterfat percentage	15	7
	Casein percentage	10	7
	Casein–Butterfat ratio	13	7
	Persistency of lactation	4	7
	Dairy merit	5	7
Hancock[30]	Grazing Time	72	6
	Loafing Time	6	6
	Lying down Time	3	6
	Distances covered	3	6
	Number of defecations	8	6
	Number of urinations	1	6
	Number of drinks	5	6
Hancock[31]	Body weights	26	10
	Absolute growth rates	13	10
	Instantaneous relative growth rate	11	10
	Linear body measurements	5–30	10
Hancock[32]	Red-cell count	4	12
	Red-cell volume	8	10
	Haemoglobin content	13	10
	Red-cell fragility	15	25
	Blood calcium	1	19
	Blood magnesium	19	19
	Blood phosphorus	15	19
	Blood sugar	1	18
	Total blood acetone bodies	1	19
	Body temperature	37	7
	Respiration rate	15	7
	Dry-matter intake (pasture)	8	12
	Faecal-nitrogen concentration	21	12
	Dry-matter content of faeces	5	12
	Milk-fat Reichert-Meissl value	11	4
	Milk-fat iodine value	4	4
	Milk-fat softening point	1	4
	Milk-fat saponification value	9	4
	Faecal egg count	2½	16
	Titre response to *Br. Abortus* vaccination	2	12
James[33]	Sperm concentration	8	12
	Total sperm	8	12
	Service volume	2	12
	Reduction factor	4	12

Carter[28] discussed the interpretation of twin efficiency values along the following lines:

(1) The efficiency value determined from a limited sample is only an estimate of a hypothetical population quantity and is therefore subject to considerable sampling variation.

(2) The sensitivity of statistical comparison of treatments depends not only on the "error" variance, but also on the "degrees of freedom" available. For example, no test of significance at all would be possible with one split-twin pair.

(3) The possibility of real environment x heredity interaction when different treatments are applied cannot be discounted. This obviously necessitates the use of a reasonable genotypic range of experimental material (certainly more than one set) if dubious extrapolation is to be avoided. Since in a non-repeated treatment-experiment, the treatment x heredity interaction is inevitably included in the "error," the estimate of error variation from a uniformity trial may well under-estimate the error appropriate to treatment comparisons, with consequent over-estimation of "E".

(4) It is probable that the genetic variability in a sample of monozygotic twins would be greater than that in a sample from a particular herd or farm. This would imply that the numerator used in the formula for "E," i.e., between-set variance, is in fact too high, leading again to an over-estimate. On the other hand, the fact that such a twin sample is representative of a wide population should justify increased confidence in generalization of experimental results.

(5) Problems of wastage also decrease the usefulness of monozygotic twins below the level indicated by the twin efficiency values. If one member of a set of twins dies or becomes useless for experimental purposes it is obvious that in most cases a co-twin must be excluded.

(6) An efficiency value can only be related to a population which is clearly specified—in regard, for example, to age of animals and length of standardized lactation period.

With due regard to the above qualifications, the twin efficiency values are nevertheless a useful concept in indicating, for most characters, the marked experimental superiority of twins over ordinary cows.

4. HERITABILITY STUDIES

The analysis of twin experiments allows a partitioning of the total variations into parts ascribable to genotype, to environment (treatment) and to genotype—environment interaction + error. The heritability coefficient derived from such analysis is related to the total genetic variance, i.e., it includes all the epistatic and dominance as well as additive gene effects.

Bonnier, Hansson and Skjervold[36] described two trials with monozygotic twins which yielded data from which the genetic contribution to growth and production could be estimated. It was

concluded that there was a tendency for the hereditary portion of variance in body measurements to increase with age. The results also indicated that the anterior measurements of the body were more strongly under hereditary control than the posterior measurements. From the analysis of the milk production data it was concluded that heredity plays a very prominent role in the expression of all productive characters and usually outweighs the effects of differences in feeding intensity, even when these differences are large. Especially such qualitative characters as the fat and protein percentages are very strongly inherited. These results were confirmed to a large extent by the results recorded in Part VIII of this series of papers[37].

Robertson[38] criticised some of the findings of Bonnier *et al.*[3] mainly on the grounds that the "hereditary" portion of variance (between-set differences) included also non-inherited effects such as common pre-natal environment, contemporaneity, and similar local environment. He concluded that it was most likely that the similarity beween twin sets within a lactation was due to the non-genetic causes listed, and not to differences between sets in rate of approach to maturity.

It may be noted that in Hancock's work[37] contemporaneity and similarity of local environment could have accounted for only very little of the portion of variance ascribed to heredity.

Thoele and Hervey[35] estimated, from trials with monozygotic twin heifers, heritability coefficients of 86–96 per cent for body weights and 94–98 per cent for height at withers.

From a uniformity trial involving 13 sets of monozygotic twins, Winzenried and Wanntorp[39] found that genetic factors strongly influenced the vitamin A and carotene levels of the milk.

Jarl[40] noted that although there was no significant difference in the ascorbic acid (Vitamin C) content between sets of twins, the differences within dizygotic twin sets were twice as great as the differences within sets of monozygotic twins. This was taken as evidence that ascorbic acid content of milk is to some extent genetically determined.

Bonnier and Hansson[41] concluded that the values of protein and of lactose attained at fixed fat percentages in milk, are mainly determined by hereditary factors. This conclusion was based on investigations involving 19 sets of monozygotic and 9 sets of dizygotic twins in addition to 2 sets of twins of uncertain zygocity.

A trial, in which it was attempted to measure the effects of different feeding levels on the sexual strength of bulls, was conducted by Bane[42] with 6 sets of monozygotic bull twins. It was concluded that intensity of feeding influenced principally the rate of physical development, but had only a slight effect on the total sperm production. As between-set variance was twelve times as great as the within-set variance, it was concluded that the sperm-producing capacity of bulls was determined by hereditary factors. Similar studies by James[33], in which the effects of a great number

of environmental factors were studied, yielded much lower estimates for the heritability of spermatic characters.

It was obvious that many trials from which heritability estimates have been attempted, suffer from certain weaknesses in design and scope which make conclusions drawn from them rather uncertain. The following few remarks on the analysis of twin experiments, from the point of view of extracting genetic information, will make this clear.

In uniformity trials, if these are not time-repeated, only two sources of variation can be distinguished: between-set differences, and within-set differences. The between-set variance contains not only all genetic effects—additive as well as those due to dominance and epistasis—but also includes any genotype x environment interaction present. Heritability estimates based on uniformity trials are therefore likely to be too high.

In split-twin experiments it is, on the other hand, possible to distinguish between three sources of variation: between-set variance, treatment variance, and error. The first term contains, as in uniformity trials, all genetic variation and that due to common pre-natal and contemporary environments. The genotype x environment interactions, however, are included in the error term. If the twin-sets used are all of the same age and have been brought up together, it is possible, in a split-twin experiment, to separate from the hereditary portion of variance all other non-genetic influences except those due to difference in pre-natal environment.

CONCLUSIONS

Since the beginning of twin research at Ruakura, work in this field has indicated that the available methods of diagnosis are sufficiently simple and reliable to make the differentiation between monozygotic and dizygotic twins a relatively easy matter. Although statistical data indicate that the frequency of monozygotic twinning is low, sufficient twins to make large-scale investigations possible should be available in dairy-farming areas in view of the fact that any dairy cattle population is to a very large extent made up of females breeding regularly once a year (see Hancock[43]). Uniformity trials and information from treatment experiments in which twins have been used have shown fairly conclusively the marked superiority of monozygotic twins over other animals as experimental material. This undoubted superiority may have induced some research workers to use so few sets of twins in their experiments that the results could not justify conclusions of general applicability. However, it seems more likely that in many cases the reason for the small number of sets used was the fact that additional sets could not be found. In view of the above discussion regarding the ease whereby monozygotic twins may be recognized and the total numbers born in a cattle population, it seems that the remedy should lie in a more efficient organisation for the collection of twins. As conditions vary greatly from country to country, it is obviously not possible to give specific advice on this matter, although the general principles outlined in the first paper of this series should apply to all conditions.

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PASTURE GROWTH AND SOIL FERTILITY

VI. INFLUENCE OF RED AND WHITE CLOVERS, SUPERPHOSPHATE, LIME, AND SHEEP GRAZING, ON PASTURE YIELDS AND COMPOSITION, AND ON THE GROWTH OF SUBSEQUENT FORAGE CROPS; RESULTS AT GORE AND LINCOLN

By P. D. Sears, J. P. Lambert and W. G. Thurston, Grasslands Division, Department of Scientific and Industrial Research

(*Received for publication, 3 August 1953*)

Summary

(1) Similar trials to those conducted at Palmerston North and reported in Parts I–IV, were carried out at Gore and Lincoln.

(2) At Gore, the pattern of pasture growth was similar to that at Palmerston North, except that the responses to superphosphate and lime were much greater. Total pasture yields were much lower than at Palmerston North at all seasons except summer when yields were similar. The differences were due mainly to greater grass yields at Palmerston North, clover production being similar.

(3) In the Gore mowing series, average nitrogen levels in the grass herbage varied from 1.96 per cent (in the cloverless plots of the N.R. treatment) to 2.57 per cent (in the grass-clover association of the F.R. treatment). The annual yields of N in the total herbage varied from 22 lb. to 324 lb. per acre. Irrespective of fertiliser treatment, N levels in the clovers were higher than those in the grasses, with white clover (average 4.29 per cent) higher than that in red clover (average 3.97 per cent). The underground transfer of N to associated grasses was much higher from white clover than from red clover.

(4) In the Gore mowing series superphosphate plus lime increased levels of Ca and P in the leaf of the grasses and clovers and also the leaf N levels of the clovers in the grass-clover combinations.

(5) It was estimated that at Gore the nitrogen fixed through the white clovers and utilised by the clover and the associated non-legumes, was only about half that at Palmerston North. Red clover was less effective than white clover in this respect.

(6) Yields and differences between the rape crops grown subsequent to the pasture treatments were similar at Gore to those at Palmerston North.

(7) At Lincoln, due to dry conditions, all pasture yields were very low and the treatment effects measured were lower than at the other two centres, although they follow the same overall pattern.

Introduction

Parts I–V of this series[1, 2, 3, 4, 5] reported results of field trials at Palmerston North measuring the effects on pasture growth and soil fertility, of clovers, superphosphate and lime, and of sheep dung and urine. In this paper are presented results from parallel trials conducted at the Gore[6] and Lincoln[7] sub-stations of the Grasslands Division during the period 1948 to 1952. It was considered desirable to conduct these parallel trials because of the different climatic conditions at the three centres. The general background to the trials to be reported is the same as at Palmerston North and no consideration of the objects of the trial is required.

All trials were similar in layout, seeds mixtures, fertiliser treatment, and grazing and cropping practices, and at each centre the experimental area was on soil considered to possess potentially high fertility.

Climate

The differences in climate between the three centres are considered, within New Zealand, to be large. Each centre, moreover, is typical of relatively large lowland regions, the climate of Palmerston North being similar to that of much of the western side of the North Island, Lincoln to that of the Canterbury Plains, and Gore to that of Southland. A general account of the climatic patterns within New Zealand has been published by Kidson[8], while more detailed regional comparisons have recently been made by Garnier[9, 10]. Adapting Thornthwaite's major climate scales, Garnier shows Palmerston North as B B'r, Lincoln at C C's and Gore as B C'r. In brief outline the climate of Palmerston North is warm and humid (annual rainfall 39 in.) with only a slight summer moisture deficiency. Lincoln has a much lower and less reliable rainfall (25 in.), greater temperature extremes, and is subject to occasional desiccating Föhn winds, all of which lead to definite summer moisture deficiency in most years. Gore has a temperature range similar to Lincoln but an even and reliable rainfall (35 in.) similar to that at Palmerston North. There is normally no summer moisture deficiency as temperatures are lower throughout the year than at Palmerston North.

A summary of rainfall and air temperature data from Gore and Lincoln during the experimental period, is shown in Table I. At Gore the conditions were reasonably normal except for two dry spells during the summer and autumn of 1949–50, and the spring and summer of 1950. At Lincoln, however, the rainfall during the first two years was abnormally low whereas for the final two years it was abnormally high. The trial, therefore, was continued over an insufficient period to give a full picture, and thus the results are of limited value.

Experimental Procedure

At both centres the experimental sites were areas of flat land adjacent to the main buildings. At Gore the soil is a recent alluvial grey sandy silt loam overlying gravels from the Mataura river[11]. The area had been used previously as a storage area for a linen flax factory; no lime or mineral fertiliser had been applied for many years. Over the area the soil profile was reasonably uniform except for a few small gravel "bars" which in some places came to within 3 to 4 in. of the surface. For most of the trial period, this lack of uniformity did not appear to have much effect on pasture growth, and the more shallow soil areas became obvious only in the dry summer and autumn of 1950-51. The Lincoln area is on a deep soil classified by the Soil Bureau as "Wakanui silt loam on sands" (Pers. Comm.), and is regarded as one of the best Canterbury types for pasture growth. The area had previously been used for cropping of oats, wheat, and peas, and for inter-cultivated single plants of timothy. At the time of sowing it was considered to be uniform in soil fertility.

At both centres the areas were cultivated during the summer and sown in the early autumn of 1948. The four main treatments were the same as in the Palmerston North series (Grazing N.R. and F.R., and Mowing N.R. and F.R.). Two extra sub-treatments were included within each main treatment to measure the separate effects of red and white clovers, giving the following series:

(1) Grasses alone — No fertiliser
(2) Grasses + clovers — No fertiliser
(3) Grasses + clovers — + superphosphate

TABLE I(a). AVERAGE AIR TEMPERATURES AND RAINFALL BY SEASONS FOR THE YEARS 1948-52 AT GORE

Season (1)	Gore (2)						
	Screen air temperatures °F					Rainfall	
	Mean Daily Max.	Mean Daily Min.	Mean Daily	Max. Recorded	Min. Recorded	Total (inches)	Departure from 10 year average 1938-48 (inches)
1948-49							
Autumn	60.5	40.3	50.6	71.0	30.0	5.77	- 2.57
Winter	51.1	34.5	42.8	59.0	24.0	5.51	- 1.38
Spring	60.0	39.9	50.0	75.3	30.0	10.55	+ 1.92
Summer	68.1	47.0	57.6	84.7	34.3	9.00	- 1.60
	59.9	40.4	50.3	72.5	29.6	30.83	- 3.63
1949-50							
Autumn	57.8	39.2	48.5	72.0	29.3	8.92	+ 0.58
Winter	50.3	35.4	42.8	60.7	27.0	9.80	+ 2.91
Spring	61.8	40.7	51.3	75.0	31.0	7.97	- 0.66
Summer	68.6	47.1	57.9	85.7	36.3	8.87	- 1.73
	59.6	40.6	50.1	73.4	30.9	35.56	+ 1.10
1950-51							
Autumn	59.9	40.4	50.2	73.3	30.0	4.06	- 4.28
Winter	49.1	33.8	41.5	57.3	24.7	7.25	+ 0.36
Spring	62.9	41.6	52.3	77.0	31.3	6.13	- 2.50
Summer	67.2	46.1	56.6	83.0	34.0	6.68	- 3.92
	59.8	40.5	50.2	72.7	30.0	24.12	-10.34
1951-52							
Autumn	61.7	40.5	51.1	76.3	28.7	8.54	+ 0.20
Winter	49.1	32.6	40.8	59.7	25.0	5.95	- 0.94
Spring	61.0	41.9	51.4	72.3	32.7	10.38	+ 1.75
Summer	66.8	46.1	56.4	81.0	35.7	11.88	+ 1.28
	59.7	40.3	49.9	72.3	30.5	36.75	+ 2.29
1948-52							
Autumn	60.0	40.1	50.1	73.2	29.5	6.82	- 1.52
Winter	49.9	34.1	42.0	59.2	25.2	7.13	+ 0.24
Spring	61.4	41.0	51.3	74.9	31.3	8.76	+ 0.13
Summer	67.7	46.6	57.1	83.6	35.1	9.11	- 1.49
	59.8	40.5	50.1	72.7	30.3	31.82	- 2.64

(1) Spring: September-November; Summer: December-February
Autumn: March-May; Winter: June-August.

(2) Recorded at East Gore, 2 miles from area.

TABLE I(b). AVERAGE AIR TEMPERATURES AND RAINFALL BY SEASONS FOR THE YEARS 1948-52 AT LINCOLN

Season (1)	Lincoln (3)						
	Screen air temperatures °F					Rainfall	
	Mean Daily Max.	Mean Daily Min.	Mean Daily	Max. Recorded	Min. Recorded	Total (inches)	Departure from 10 year average 1938-48 (inches)
1948-49							
Autumn	62.4	43.4	52.9	84.6	28.7	5.58	- 0.99
Winter	50.8	34.6	42.7	64.2	24.6	3.15	- 4.25
Spring	61.4	41.5	51.5	81.2	29.6	5.78	+ 0.13
Summer	72.9	49.0	60.9	94.7	32.5	3.05	- 3.25
	61.9	42.1	52.0	94.7	24.6	17.56	- 8.36
1949-50							
Autumn	61.2	41.9	51.6	86.8	25.9	5.07	- 1.50
Winter	52.4	34.0	43.2	66.4	22.1	5.21	- 2.19
Spring	63.1	41.8	52.9	83.4	19.4	2.46	- 3.19
Summer	71.2	49.2	60.2	89.0	33.7	7.57	+ 1.27
	62.2	41.7	52.0	89.0	19.4	20.31	- 5.61
1950-51							
Autumn	61.5	44.1	52.8	85.6	28.5	4.62	- 1.95
Winter	50.8	32.2	41.5	61.4	21.6	9.46	+ 2.06
Spring	61.9	42.1	52.0	82.9	28.0	6.59	+ 0.94
Summer	67.8	48.7	58.3	87.8	35.0	14.48	+ 8.18
	60.5	41.8	51.2	87.8	21.6	35.15	+ 9.23
1951-52							
Autumn	60.6	44.0	52.3	80.8	24.1	14.12	+ 7.55
Winter	51.0	32.9	41.9	64.5	22.0	5.14	- 2.26
Spring	61.7	40.8	51.3	79.2	27.1	5.49	- 0.16
Summer	69.6	47.3	58.5	89.0	33.2	6.25	- 0.05
	60.7	41.2	51.0	89.0	22.0	31.00	+ 5.08
1948-52							
Autumn	61.4	43.3	52.4	86.8	24.1	7.35	+ 0.78
Winter	51.2	33.4	42.3	66.4	21.6	5.74	- 1.66
Spring	62.3	41.5	51.9	83.4	19.4	5.08	- 0.57
Summer	70.4	48.5	59.5	94.7	32.5	7.84	+ 1.54
	61.3	41.7	51.5	94.7	19.4	26.01	+ 0.09

(3) Recorded at Lincoln College.

(4) Grasses + clovers + lime
(5) Grasses + clovers + superphosphate + lime
(6) Grasses + red clover + superphosphate + lime
(7) Grasses + white clover + superphosphate + lime
(8) Grasses alone + superphosphate + lime

The seed mixtures, fertiliser applications, fencing, herbage measurement and analysis techniques, and the collection and return of dung and urine programmes conformed to the same pattern as that in the Palmerston North trial. The sole differences in procedure related to fertiliser applications, and the return of urine to the F.R. series.

Carter[28] discussed the interpretation of twin efficiency values along the following lines:

(1) The efficiency value determined from a limited sample is only an estimate of a hypothetical population quantity and is therefore subject to considerable sampling variation.

(2) The sensitivity of statistical comparison of treatments depends not only on the "error" variance, but also on the "degrees of freedom" available. For example, no test of significance at all would be possible with one split-twin pair.

(3) The possibility of real environment x heredity interaction when different treatments are applied cannot be discounted. This obviously necessitates the use of a reasonable genotypic range of experimental material (certainly more than one set) if dubious extrapolation is to be avoided. Since in a non-repeated treatment-experiment, the treatment x heredity interaction is inevitably included in the "error," the estimate of error variation from a uniformity trial may well under-estimate the error appropriate to treatment comparisons, with consequent over-estimation of "E".

(4) It is probable that the genetic variability in a sample of monozygotic twins would be greater than that in a sample from a particular herd or farm. This would imply that the numerator used in the formula for "E," i.e., between-set variance, is in fact too high, leading again to an over-estimate. On the other hand, the fact that such a twin sample is representative of a wide population should justify increased confidence in generalization of experimental results.

(5) Problems of wastage also decrease the usefulness of monozygotic twins below the level indicated by the twin efficiency values. If one member of a set of twins dies or becomes useless for experimental purposes it is obvious that in most cases a co-twin must be excluded.

(6) An efficiency value can only be related to a population which is clearly specified—in regard, for example, to age of animals and length of standardized lactation period.

With due regard to the above qualifications, the twin efficiency values are nevertheless a useful concept in indicating, for most characters, the marked experimental superiority of twins over ordinary cows.

4. HERITABILITY STUDIES

The analysis of twin experiments allows a partitioning of the total variations into parts ascribable to genotype, to environment (treatment), and to genotype—environment interaction + error. The heritability coefficient derived from such analysis is related to the total genetic variance, i.e., it includes all the epistatic and dominance as well as additive gene effects.

Bonnier, Hansson and Skjervold[36] described two trials with monozygotic twins which yielded data from which the genetic contribution to growth and production could be estimated. It was

concluded that there was a tendency for the hereditary portion of variance in body measurements to increase with age. The results also indicated that the anterior measurements of the body were more strongly under hereditary control than the posterior measurements. From the analysis of the milk production data it was concluded that heredity plays a very prominent role in the expression of all productive characters and usually outweighs the effects of differences in feeding intensity, even when these differences are large. Especially such qualitative characters as the fat and protein percentages are very strongly inherited. These results were confirmed to a large extent by the results recorded in Part VIII of this series of papers[37].

Robertson[38] criticised some of the findings of Bonnier *et al.*[3] mainly on the grounds that the "hereditary" portion of variance (between-set differences) included also non-inherited effects such as common pre-natal environment, contemporaneity, and similar local environment. He concluded that it was most likely that the similarity beween twin sets within a lactation was due to the non-genetic causes listed, and not to differences between sets in rate of approach to maturity.

It may be noted that in Hancock's work[37] contemporaneity and similarity of local environment could have accounted for only very little of the portion of variance ascribed to heredity.

Thoele and Hervey[35] estimated, from trials with monozygotic twin heifers, heritability coefficients of 86–96 per cent for body weights and 94–98 per cent for height at withers.

From a uniformity trial involving 13 sets of monozygotic twins, Winzenried and Wanntorp[39] found that genetic factors strongly influenced the vitamin A and carotene levels of the milk.

Jarl[40] noted that although there was no significant difference in the ascorbic acid (Vitamin C) content between sets of twins, the differences within dizygotic twin sets were twice as great as the differences within sets of monozygotic twins. This was taken as evidence that ascorbic acid content of milk is to some extent genetically determined.

Bonnier and Hansson[41] concluded that the values of protein and of lactose attained at fixed fat percentages in milk, are mainly determined by hereditary factors. This conclusion was based on investigations involving 19 sets of monozygotic and 9 sets of dizygotic twins in addition to 2 sets of twins of uncertain zygocity.

A trial, in which it was attempted to measure the effects of different feeding levels on the sexual strength of bulls, was conducted by Bane[42] with 6 sets of monozygotic bull twins. It was concluded that intensity of feeding influenced principally the rate of physical development, but had only a slight effect on the total sperm production. As between-set variance was twelve times as great as the within-set variance, it was concluded that the sperm-producing capacity of bulls was determined by hereditary factors. Similar studies by James[33], in which the effects of a great number

of environmental factors were studied, yielded much lower estimates for the heritability of spermatic characters.

It was obvious that many trials from which heritability estimates have been attempted, suffer from certain weaknesses in design and scope which make conclusions drawn from them rather uncertain. The following few remarks on the analysis of twin experiments, from the point of view of extracting genetic information, will make this clear.

In uniformity trials, if these are not time-repeated, only two sources of variation can be distinguished: between-set differences, and within-set differences. The between-set variance contains not only all genetic effects—additive as well as those due to dominance and epistasis—but also includes any genotype x environment interaction present. Heritability estimates based on uniformity trials are therefore likely to be too high.

In split-twin experiments it is, on the other hand, possible to distinguish between three sources of variation: between-set variance, treatment variance, and error. The first term contains, as in uniformity trials, all genetic variation and that due to common pre-natal and contemporary environments. The genotype x environment interactions, however, are included in the error term. If the twin-sets used are all of the same age and have been brought up together, it is possible, in a split-twin experiment, to separate from the hereditary portion of variance all other non-genetic influences except those due to difference in pre-natal environment.

CONCLUSIONS

Since the beginning of twin research at Ruakura, work in this field has indicated that the available methods of diagnosis are sufficiently simple and reliable to make the differentiation between monozygotic and dizygotic twins a relatively easy matter. Although statistical data indicate that the frequency of monozygotic twinning is low, sufficient twins to make large-scale investigations possible should be available in dairy-farming areas in view of the fact that any dairy cattle population is to a very large extent made up of females breeding regularly once a year (see Hancock[43]). Uniformity trials and information from treatment experiments in which twins have been used have shown fairly conclusively the marked superiority of monozygotic twins over other animals as experimental material. This undoubted superiority may have induced some research workers to use so few sets of twins in their experiments that the results could not justify conclusions of general applicability. However, it seems more likely that in many cases the reason for the small number of sets used was the fact that additional sets could not be found. In view of the above discussion regarding the ease whereby monozygotic twins may be recognized and the total numbers born in a cattle population, it seems that the remedy should lie in a more efficient organisation for the collection of twins. As conditions vary greatly from country to country, it is obviously not possible to give specific advice on this matter, although the general principles outlined in the first paper of this series should apply to all conditions.

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All trials were similar in layout, seeds mixtures, fertiliser treatment, and grazing and cropping practices, and at each centre the experimental area was on soil considered to possess potentially high fertility.

Climate

The differences in climate between the three centres are considered, within New Zealand, to be large. Each centre, moreover, is typical of relatively large lowland regions, the climate of Palmerston North being similar to that of much of the western side of the North Island, Lincoln to that of the Canterbury Plains, and Gore to that of Southland. A general account of the climatic patterns within New Zealand has been published by Kidson[8], while more detailed regional comparisons have recently been made by Garnier[9, 10]. Adapting Thornthwaite's major climate scales, Garnier shows Palmerston North as B B'r, Lincoln at C C's and Gore as B C'r. In brief outline the climate of Palmerston North is warm and humid (annual rainfall 39 in.) with only a slight summer moisture deficiency. Lincoln has a much lower and less reliable rainfall (25 in.), greater temperature extremes, and is subject to occasional desiccating Föhn winds, all of which lead to definite summer moisture deficiency in most years. Gore has a temperature range similar to Lincoln but an even and reliable rainfall (35 in.) similar to that at Palmerston North. There is normally no summer moisture deficiency as temperatures are lower throughout the year than at Palmerston North.

A summary of rainfall and air temperature data from Gore and Lincoln during the experimental period, is shown in Table I. At Gore the conditions were reasonably normal except for two dry spells during the summer and autumn of 1949–50, and the spring and summer of 1950. At Lincoln, however, the rainfall during the first two years was abnormally low whereas for the final two years it was abnormally high. The trial, therefore, was continued over an insufficient period to give a full picture, and thus the results are of limited value.

Experimental Procedure

At both centres the experimental sites were areas of flat land adjacent to the main buildings. At Gore the soil is a recent alluvial grey sandy silt loam overlying gravels from the Mataura river[11]. The area had been used previously as a storage area for a linen flax factory; no lime or mineral fertiliser had been applied for many years. Over the area the soil profile was reasonably uniform except for a few small gravel "bars" which in some places came to within 3 to 4 in. of the surface. For most of the trial period, this lack of uniformity did not appear to have much effect on pasture growth, and the more shallow soil areas became obvious only in the dry summer and autumn of 1950-51. The Lincoln area is on a deep soil classified by the Soil Bureau as "Wakanui silt loam on sands" (Pers. Comm.), and is regarded as one of the best Canterbury types for pasture growth. The area had previously been used for cropping of oats, wheat, and peas, and for inter-cultivated single plants of timothy. At the time of sowing it was considered to be uniform in soil fertility.

At both centres the areas were cultivated during the summer and sown in the early autumn of 1948. The four main treatments were the same as in the Palmerston North series (Grazing N.R. and F.R., and Mowing N.R. and F.R.). Two extra sub-treatments were included within each main treatment to measure the separate effects of red and white clovers, giving the following series:

(1) Grasses alone — No fertiliser
(2) Grasses + clovers — No fertiliser
(3) Grasses + clovers — + superphosphate

TABLE I(a). AVERAGE AIR TEMPERATURES AND RAINFALL BY SEASONS FOR THE YEARS 1948-52 AT GORE

Season (1)	Gore (2)						
	Screen air temperatures °F					Rainfall	
	Mean Daily Max.	Mean Daily Min.	Mean Daily	Max. Recorded	Min. Recorded	Total (inches)	Departure from 10 year average 1938-48 (inches)
1948-49							
Autumn	60.5	40.3	50.6	71.0	30.0	5.77	- 2.57
Winter	51.1	34.5	42.8	59.0	24.0	5.51	- 1.38
Spring	60.0	39.9	50.0	75.3	30.0	10.55	+ 1.92
Summer	68.1	47.0	57.6	84.7	34.3	9.00	- 1.60
	59.9	40.4	50.3	72.5	29.6	30.83	- 3.63
1949-50							
Autumn	57.8	39.2	48.5	72.0	29.3	8.92	+ 0.58
Winter	50.3	35.4	42.8	60.7	27.0	9.80	+ 2.91
Spring	61.8	40.7	51.3	75.0	31.0	7.97	- 0.66
Summer	68.6	47.1	57.9	85.7	36.3	8.87	- 1.73
	59.6	40.6	50.1	73.4	30.9	35.56	+ 1.10
1950-51							
Autumn	59.9	40.4	50.2	73.3	30.0	4.06	- 4.28
Winter	49.1	33.8	41.5	57.3	24.7	7.25	+ 0.36
Spring	62.9	41.6	52.3	77.0	31.3	6.13	- 2.50
Summer	67.2	46.1	56.6	83.0	34.0	6.68	- 3.92
	59.8	40.5	50.2	72.7	30.0	24.12	-10.34
1951-52							
Autumn	61.7	40.5	51.1	76.3	28.7	8.54	+ 0.20
Winter	49.1	32.6	40.8	59.7	25.0	5.95	- 0.94
Spring	61.0	41.9	51.4	72.3	32.7	10.38	+ 1.75
Summer	66.8	46.1	56.4	81.0	35.7	11.88	+ 1.28
	59.7	40.3	49.9	72.3	30.5	36.75	+ 2.29
1948-52							
Autumn	60.0	40.1	50.1	73.2	29.5	6.82	- 1.52
Winter	49.9	34.1	42.0	59.2	25.2	7.13	+ 0.24
Spring	61.4	41.0	51.3	74.9	31.3	8.76	+ 0.13
Summer	67.7	46.6	57.1	83.6	35.1	9.11	- 1.49
	59.8	40.5	50.1	72.7	30.3	31.82	- 2.64

(1) Spring: September-November; Summer: December-February
Autumn: March-May; Winter: June-August.

(2) Recorded at East Gore, 2 miles from area.

TABLE I(b). AVERAGE AIR TEMPERATURES AND RAINFALL BY SEASONS FOR THE YEARS 1948-52 AT LINCOLN

Season (1)	Lincoln (3)						
	Screen air temperatures °F					Rainfall	
	Mean Daily Max.	Mean Daily Min.	Mean Daily	Max. Recorded	Min. Recorded	Total (inches)	Departure from 10 year average 1938-48 (inches)
1948-49							
Autumn	62.4	43.4	52.9	84.6	28.7	5.58	- 0.99
Winter	50.8	34.6	42.7	64.2	24.6	3.15	- 4.25
Spring	61.4	41.5	51.5	81.2	29.6	5.78	+ 0.13
Summer	72.9	49.0	60.9	94.7	32.5	3.05	- 3.25
	61.9	42.1	52.0	94.7	24.6	17.56	- 8.36
1949-50							
Autumn	61.2	41.9	51.6	86.8	25.9	5.07	- 1.50
Winter	52.4	34.0	43.2	66.4	22.1	5.21	- 2.19
Spring	64.1	41.8	52.9	83.4	19.4	2.46	- 3.19
Summer	71.2	49.2	60.2	89.0	33.7	7.57	+ 1.27
	62.2	41.7	52.0	89.0	19.4	20.31	- 5.61
1950-51							
Autumn	61.5	44.1	52.8	85.6	28.5	4.62	- 1.95
Winter	50.8	32.2	41.5	61.4	21.6	9.48	+ 2.06
Spring	61.9	42.1	52.0	82.9	25.0	6.59	+ 0.94
Summer	67.6	48.7	58.3	87.8	35.0	14.48	+ 8.18
	60.5	41.8	51.2	87.8	21.6	35.17	+ 9.23
1951-52							
Autumn	60.6	44.0	52.3	80.8	24.1	14.12	+ 7.55
Winter	51.9	32.9	41.9	64.5	22.0	5.14	- 2.26
Spring	61.7	40.8	51.3	79.2	27.1	5.49	- 0.16
Summer	69.6	47.3	58.5	89.0	33.2	6.25	- 0.05
	60.7	41.2	51.0	89.0	22.0	31.00	+ 5.08
1948-52							
Autumn	61.4	43.3	52.4	86.8	24.1	7.35	+ 0.78
Winter	51.2	33.4	42.3	66.4	21.6	5.74	- 1.66
Spring	62.3	41.5	51.9	83.4	19.4	5.08	- 0.57
Summer	70.4	48.5	59.5	94.7	32.5	7.84	+ 1.54
	61.3	41.7	51.5	94.7	19.4	26.01	+ 0.09

(3) Recorded at Lincoln College.

(4) Grasses + clovers + lime
(5) Grasses + clovers + superphosphate + lime
(6) Grasses + red clover + superphosphate + lime
(7) Grasses + white clover + superphosphate + lime
(8) Grasses alone + superphosphate + lime

The seed mixtures, fertiliser applications, fencing, herbage measurement and analysis techniques, and the collection and return of dung and urine programmes conformed to the same pattern as that in the Palmerston North trial. The sole differences in procedure related to fertiliser applications, and the return of urine to the F.R. series.

C. Chemical Composition of Pasture Herbage

Tables IV and V are a summary of the weighted results from four sets of chemical analyses made on the bulked herbage from selected treatments on the mowing series at Gore during the final two years of the pasture trial. Table IV shows percentages of N, Ca, and P, and Table V presents the calculated average weights of these elements removed annually in the herbage.

The results in Tables IV and V are discussed below in terms of individual elements.

Nitrogen: In sub-treatment 8 (grasses without clovers) the average N content of the grasses was 1.96 per cent in the N.R., and 2.31 per cent in the F.R. series. The average N content of the grasses in the grass-clover combinations was 2.53 per cent in the N.R. series, and 2.57 per cent in the F.R. series. In both the N.R. and the F.R. series, the N content of the grasses growing in association with white clover, was higher than that of the grasses growing in association with the red clover, e.g., an average of 2.64 per cent compared with 2.48 per cent. In all cases, the N content of the white clover herbage was higher than that of the red clover, e.g., an average of 4.29 per cent compared with 3.97 per cent. The application of superphosphate and lime resulted in higher N levels in both the clovers, but there was no difference in the N levels of the associated grasses. As a result there were considerable differences in the N levels of the total herbage. Thus, in the N.R. series, there was an increase from 1.96 per cent to 3.70 per cent in N. level of the total herbage due to the clovers, while in the F.R. series there was likewise an increase from 2.31 to 3.35 per cent.

The data for annual nutrient uptakes shown in Table V follow a pattern similar to that recorded at Palmerston North, although the amounts at Gore are only about one half those recorded at Palmerston North. It can be seen that this is due both to lower grass production and to lower grass N levels at Gore than at Palmerston North. Grass growth throughout at Gore was only about one half that at Palmerston North, while the N levels in Sub-treatments 2, 5, and 8 at Gore averaged 2.42 per cent, compared with an average of 3.42 per cent at Palmerston North. The difference between the clovers at the two centres was not so marked, either in total growth or in leaf N levels, although again the advantage was in favour of the Palmerston North production.

By comparing the data of Sub-treatments 6, 7, and 8 in the N.R. series it is seen that the nitrogen increase from the white clover, and taken up by the herbage, amounted to 226 lb. per acre with an increased nitrogen uptake by the grasses of 55 lb. per acre. With red clover, the total nitrogen increase in the herbage was 219 lb. per acre with an increase in the grasses of 23 lb. per acre, less than half that from the white clover. In the F.R. series the difference in annual N turnover in the pasture—animal manure cycle, due to the clovers, was 253 lb. with white clover, and 157 lb. with red. It will be seen that the pattern of species growth response to the dung and urine return was less marked than in the Palmerston North trial. The extra uptake of N by the grasses

TABLE IV. CHEMICAL COMPOSITION OF THE HERBAGE FROM THE MOWING SERIES AT GORE FOR THE PERIOD 1st SEPTEMBER 1949 TO 31st AUGUST 1951 (% of Dry Matter)

Sub-treatment	Nitrogen				Calcium				Phosphorus			
	Grasses	Red Clover	White Clover	Total Herbage	Grasses	Red Clover	White Clover	Total Herbage	Grasses	Red Clover	White Clover	Total Herbage
2 N.R.	2.56	3.86	4.29	3.66	0.39	1.41	1.21	0.99	0.30	0.29	0.29	0.30
5 "	2.55	4.19	4.54	3.67	0.55	1.67	1.48	1.15	0.35	0.37	0.34	0.34
6 "	2.49	4.23	-	3.73	*	1.52	-	*	*	0.36	-	*
7 "	2.54	-	4.48	3.70	*	-	1.42	*	*	-	0.34	*
8 "	1.96	-	-	1.96	*	-	-	*	*	-	-	*
2 F.R.	2.58	3.65	4.02	3.26	0.44	1.25	1.19	0.91	0.34	0.31	0.29	0.31
5 "	2.59	3.93	4.27	3.39	0.52	1.34	1.46	0.97	0.40	0.36	0.37	0.38
6 "	2.47	3.97	-	3.21	*	1.49	-	*	*	0.35	-	*
7 "	2.65	-	4.14	3.31	*	-	1.44	*	*	-	0.37	*
8 "	2.31	-	-	2.31	*	-	-	*	*	-	-	*

* not determined

TABLE V. MEAN ANNUAL YIELDS OF ELEMENTS IN THE HERBAGE FROM THE MOWING SERIES AT GORE DURING THE PERIOD 1st SEPTEMBER 1949 TO 31st AUGUST 1951 (lb per acre)

Sub-Treatment	Dry Matter				Nitrogen				Calcium				Phosphorus			
	Grasses	Red Clover	White Clover	Total Herbage	Grasses	Red Clover	White Clover	Total Herbage	Grasses	Red Clover	White Clover	Total Herbage	Grasses	Red Clover	White Clover	Total Herbage
2 N.R.	2178	1173	3403	6754	56	45	146	247	9	17	42	68	7	4	10	21
5 "	2733	1417	2669	6819	70	60	121	251	15	24	40	79	10	5	9	24
6 "	1830	4629	-	6459	45	196	-	241	*	71	-	*	*	17	-	*
7 "	3026	-	3816	6842	77	-	171	248	*	-	54	*	*	-	13	*
8 "	1128	-	-	1128	22	-	-	22	*	-	-	*	*	-	-	*
2 F.R.	4270	367	3518	8155	110	14	142	266	18	5	42	65	15	1	10	26
5 "	4877	605	3992	9474	126	24	170	320	25	8	58	91	20	2	15	37
6 "	3582	3513	-	7095	88	140	-	228	*	53	-	*	*	13	-	*
7 "	5392	-	4362	9754	143	-	181	324	*	-	63	*	*	-	16	*
8 "	3070	-	-	3070	71	-	-	71	*	-	-	*	*	-	-	*

* not determined

in the grass + clover mixtures of the F.R. series was not so great at Gore. This could be due to: (a) the return of the bulked urine on the basis of D.M. yields rather than total N in the herbage, probably gave Sub-treatment 8 a higher return of urinary nitrogen than it should; (b) the white clover may have been utilising more of the nitrogen in the dung and urine than at Palmerston North, during the cold periods, when *Rhizobia* activity would be reduced to a greater extent than the plant's potential for growth; (c) in view of the continued grass and clover response to superphosphate and lime and to dung and urine, it is possible that nutrients other than N were also limiting factors in the grass-clover combinations.

Calcium: As in the Palmerston North trials and many others the calcium levels in the clovers were much higher than in the grasses, the levels in the clovers being approximately three times those of the grasses. In both the clovers and the grasses there were marked and consistent increases in the Ca levels following the lime + superphosphate applications. In the fertilised series, the levels in grasses and clovers were similar to those recorded at Palmerston North, and this, together with the fact that the return of dung gave no Ca increase, suggests that Ca was not a limiting factor in the fertilised sub-treatments. There was no consistent difference between the Ca levels of the red and white clovers.

Phosphorus: In both clovers and grasses there were marked responses in herbage content of P from superphosphate + lime, with an increase in the total herbage from 0.30 to 0.34 per cent in the N.R. series, and from 0.31 to 0.38 per cent P in the F.R. series. The levels are all much lower than at Palmerston North, and this, together with the continuing response to superphosphate in both the N.R. and F.R. series, strongly suggests that the phosphate cycle was not closed by the amounts of superphosphate used and by the return of dung and urine.

D. Yields of rape and ryegrass crops: Gore

No consistent difference in soil structure was observed at either ploughing in the Gore trial. In the earlier stages of the rape crop growth, there was a lighter green leaf-colour in the pure grass sub-treatments of the N.R. series than in all others, but at the time of the first measurement, leaf colour differences were much less. At this time, the leaf margins on all plots were turning purple, the colour being more pronounced in the N.R. series. The growth was taller in Sub-treatments 3, 5, and 7 than the others within each series. For the re-growth period to the second cut, all visible differences between treatments were much less although Sub-treatments 3, 5, and 7 again had slightly taller growth.

Table VI presents the separate and total yields of the first and second rape cuts. For an approximation of the significance of the yield differences in the first rape cut, the total data were analysed as though the layout was strictly randomised with five replicates. On this basis the differences required at the 5 per cent level are 1,018 lb./ac. in the N.R. series, and 925 lb./ac. in the F.R. series. Caution is, of course, required in applying these to borderline differences. However, from the yield data the following were clearcut:

TABLE VI. YIELDS OF RAPE GROWN SUBSEQUENT TO THE PASTURE TRIALS AT GORE AND LINCOLN (lb. D.M. / acre)

Main Treatment	Sub-Treatment	Gore			Lincoln
		1st Cut	2nd Cut	Total	Total
No Return Dung and Urine	1	2520	1740	4260	3290
	2	2950	1270	4220	3970
	3	5720	1870	7590	4390
	4	3950	1910	5860	4270
	5	5990	2170	8160	4740
	6	5440	1960	7300	4580
	7	6140	2090	8230	5040
	8	2640	1520	4160	2870
Full Return Dung and Urine	1	4750	2020	6770	3520
	2	5060	2120	7180	4600
	3	6890	2580	9470	5190
	4	6080	2530	8610	4250
	5	7030	2720	9750	4560
	6	7170	2640	9810	4550
	7	7470	2740	10210	4790
	8	5840	2420	8260	3810

(1) In the first cut on the N.R. series, the greatest yields were obtained on the grass + clover + superphosphate sub-treatments; then followed the limed grass + clover sub-treatments, with the lowest yielding being a group (within which there were no differences) comprising the unfertilised grass + clover, and both the cloverless sub-treatments.

(2) In the first cut on the F.R. series, yields were consistently greater than in the N.R. series but the differences between sub-treatments were lower. The only exception to a pattern of differences similar to those in the N.R. series is that the yield in the cloverless sub-treatment plus superphosphate and lime, was significantly greater than in the cloverless treatment without fertiliser.

(3) In the second cuts, there were again consistently higher yields in the F.R. then in the N.R. series and within both series there were differences in the same direction as in the first cuts. All yields were, however, much lower, and the differences much less, than in the first cuts.

(4) The range of yields in both cuts on the rape crops was similar to that obtained in the crops grown at Palmerston North after the grazing series.

(5) In the ryegrass crops grown after the rape, there were no real differences between sub-treatment yields within the N.R. and F.R. series, but the yields in the F.R. series were slightly higher throughout than in their counterparts of the N.R. series. The data are not presented in detail but the mean yield of the total of the four cuts of the ryegrass in the N.R. series was 3,390 lb. D.M. per acre (range—3,200 lb. to 3,670 lb.), and in the F.R. series was 3,970 lb. D.M. per acre (range—3,680 lb. to 4,230 lb.).

Results and Discussion: Lincoln

A. Total Dry Matter Production and Yields of Individual Species

Table VII presents the D.M. yields for all treatments, and the mean species yields from the combined mowing and grazing series in the Lincoln trial. The data shown are again for two distinct periods, the first being for the first 2½ years after sowing, during which time there was poor grass growth in all plots and only a very slow establishment of the clovers. The second period is the final year of the pasture trial, which was continued only on the mowing series.

The following cover the main points shown in Table VII.

(1) In the first period in the N.R. series, the only differences in total yield between sub-treatments were the increases resulting from the small amounts of clover (mainly red) in the grass–clover mixtures. Ryegrass was the major contributor in all sub-treatments.

(2) In the first period in the F.R. series, the grass productions in all sub-treatments were greater, and the clover yields lower than in their counterparts in the N.R. series. The net result was an increase of approximately 30 per cent in total herbage from dung and urine return. Within the F.R. series for this period, there was no consistent effect from the clovers or from the superphosphate or lime. Total yields were all very low, however, and the primary limitation in all series was, no doubt, soil moisture. It is of interest in this regard to compare the yields with those of a previous pasture trial conducted on an adjacent area, during a relatively wet period[13]. In that trial a mixture of ryegrass and red and white clover, given fertiliser and grazing similar to those in Sub-treatment 5 F.R. of this trial, produced, during its first 20 months only, over 17,000 lb. D.M. per acre. There is an even greater difference between the yields of the comparable N.R. treatments, due to the much earlier establishment and vigorous clover growth in the previous trial.

(3) In the second period in the N.R. series, there was much greater clover growth, but only a small increase in grass growth. This extra clover growth, in the main, affected total yields only by its direct contribution. This was particularly so with the red

TABLE VII. TOTAL PASTURE PRODUCTION AND YIELDS OF INDIVIDUAL SPECIES AT LINCOLN
(Yields in lb. D.M./acre in full type, percentage composition in parenthesis)

Main Treatment	Period	Sub-treatment	Yields (lb. D.M./acre)			Species yields and percentages of total yields					Main Treatment	Period	Sub-treatment	Yields (lb. D.M./acre)			Species yields and percentages of total yields				
			Mowing	*1 Grazing	Average	Ryegrass	Other Grasses	Red Clover	White Clover	Other Species				Mowing	*1 Grazing	Average	Ryegrass	Other Grasses	Red Clover	White Clover	Other Species
No Return of Dung and Urine	March 1948 (Sowing) to end of August 1950 (2½ years)	1	3860	2420	3000	2660 (88)	170 (6)	–	–	170 (6)	Proportional Return of Dung and Urine	March 1948 (sowing) to end of August 1950 (2½ years)	1	5560	2890	4000	3250 (82)	520 (13)	–	–	200 (5)
		2	4880	2260	3500	2600 (74)	170 (5)	490 (14)	Tr	240 (7)			2	6400	3340	4550	3650 (80)	370 (8)	320 (7)	50 (1)	180 (4)
		3	4710	2510	3400	2680 (79)	170 (5)	310 (9)	30 (1)	210 (6)			3	6800	3480	4800	4090 (85)	340 (7)	240 (5)	Tr	140 (3)
		4	4750	2430	3350	2580 (77)	170 (5)	300 (9)	30 (1)	270 (8)			4	6250	2760	4150	3450 (83)	360 (9)	170 (4)	Tr	170 (4)
		5	4800	2710	3550	2730 (77)	180 (5)	360 (10)	70 (2)	210 (6)			5	6780	3640	4900	4200 (86)	340 (7)	190 (4)	50 (1)	100 (2)
		6	4840	2760	3550	2600 (73)	320 (9)	430 (12)	–	210 (6)			6	5940	3220	4300	3750 (87)	260 (6)	180 (4)	–	130 (3)
		7	4310	2470	3200	2630 (82)	220 (7)	–	130 (4)	220 (7)			7	6250	4000	4900	4510 (92)	250 (5)	–	50 (1)	100 (2)
		8	3990	2360	3000	2670 (89)	210 (7)	–	–	120 (4)			8	6300	3420	4500	4770 (87)	550 (10)	–	–	160 (3)
	September 1950 to end of August 1951 (1 year)	1	1243	–	–	659 (53)	426 (34)	–	–	158 (13)		September 1950 to end of August 1951 (1 year)	1	3057	–	–	884 (29)	1987 (65)	–	–	186 (6)
		2	4820	–	–	781 (16)	331 (7)	2178 (45)	1444 (30)	83 (2)			2	6508	–	–	1318 (22)	1032 (14)	2438 (37)	1596 (25)	124 (2)
		3	5383	–	–	820 (15)	219 (4)	1945 (36)	2352 (44)	47 (1)			3	7016	–	–	1853 (26)	641 (9)	2727 (39)	1727 (25)	68 (1)
		4	5960	–	–	799 (13)	376 (6)	2584 (44)	2112 (35)	89 (2)			4	6960	–	–	1348 (19)	1520 (22)	2050 (29)	1848 (27)	194 (3)
		5	6489	–	–	1235 (19)	88 (1)	2248 (35)	2839 (44)	89 (1)			5	7540	–	–	2192 (29)	853 (11)	1910 (26)	2487 (33)	98 (1)
		6	4629	–	–	847 (18)	25 Tr	3679 (80)	Tr –	78 (2)			6	5811	–	–	1787 (31)	645 (10)	3171 (55)	149 (3)	59 (1)
		7	6853	–	–	1181 (17)	12 Tr	–	5546 (81)	126 (2)			7	7202	–	–	2288 (32)	882 (12)	–	3868 (54)	164 (2)
		8	1224	–	–	596 (49)	476 (39)	–	–	152 (12)			8	2799	–	–	1833 (66)	877 (31)	–	–	89 (3)

*1 Does not include growth during grazing periods

clover, which gave no obvious stimulus to the associated grasses. On the other hand, there was some increase in ryegrass when growing in association with white clover in those sub-treatments which received both superphosphate and lime, although neither additive alone increased ryegrass growth. Superphosphate consistently increased the growth of the white clover, but depressed the red clover in the mixtures. Lime increased the growth of both red and white clover. The combination of lime and superphosphate gave the greatest total clover production. An added complication was the reduction in the growth of other grasses, viz. Yorkshire fog, cocksfoot, and browntop, in the grass clover plots receiving both lime and superphosphate. There was no yield response to lime and superphosphate on the cloverless pastures, which both finished as open and stunted associations dominated by brown top, with small amounts of Yorkshire fog, cocksfoot, ryegrass, and flat weeds.

(4) In the second period, in the F.R. series total yields were all greater than in the N.R. series. As in the first period, this resulted largely from the extra growth of grass, the clover growth on the average being slightly less. Clovers had very little effect on the growth of the associated grass although in this respect white clover appeared to produce a small stimulus. Within sub-treatments containing both the clovers, superphosphate appears to have resulted in a slight increase in total clover growth. There was no response to lime. In all sub-treatments, the application of superphosphate appears to have stimulated ryegrass and depressed other grasses.

B. Yields of Rape and Ryegrass Crops: Lincoln

At the first ploughing after the pasture trial at Lincoln the soil texture in the grass–clover sub-treatments appeared to be much better than in the cloverless plots, the soil of which was very hard and compact. Establishment of the rape was good in all plots but early growth was slow because of dry soil conditions. In Sub-treatments 1, 8, and 6, and especially in the N.R. series, early growth was slowest with reddish-brown leaf coloration persisting throughout the trial. Leaf colour in all other sub-treatments remained dark-green throughout. A small amount of aphis attack occurred on Sub-treatments 1 and 8 N.R. with a minor amount on the corresponding F.R. sub-treatments. At the time of cutting (mid-February) there were marked differences in height and colour. Sub-treatments 1 and 8 N.R. had growth about 6–7 in. high and with many reddish brown and dead leaves. In Sub-treatments 1 and 8 F.R., the growth was up to 10 in. high with less reddish-brown and purple leaf coloration. In Sub-treatments 3, 5, and 7 N.R. and F.R., the crops were about 14–15 in. high with dark-green leaves, having only very little purple coloration on the leaf margins.

Table VI presents the mean total D.M. yields of the rape crops. The differences between treatments followed the same general pattern as in the Gore and Palmerston North trial, although the total yields were lower throughout, and the range of the yield

differences was less. An exception to this close agreement was that the yields of the limed grass–clover treatments in the F.R. series all appeared to be lower than their unlimed counterparts. These differences were all very small, however, and of doubtful significance. The only really consistent differences were the much greater crop yields on the grass + clover + superphosphate sub-treatments as compared with the cloverless associations with or without superphosphate and lime. With the Italian ryegrass yields (not presented in detail), there was no real difference in the totals from the four cuts, although there were actually small visible differences in the same direction as for the rape yields, in the first two cuts. The mean total ryegrass yield from all treatments was 2,340 lb. D.M. per acre (range: 2,000 to 2,570 lb.).

Conclusions

The results from these trials at Lincoln and Gore show the same general pattern of improvement in soil fertility due to the presence of clovers and to the return of dung and urine, as was demonstrated in the Palmerston North trial. There are, however, some points of difference due to climate and to differences in soil nutrient levels at the three centres.

There seems little doubt that, at Lincoln, the total growth and development of soil fertility were limited by the early severe soil moisture deficiency. At Gore, on the other hand, climatic conditions were more favourable. At both centres, the responses to lime and superphosphate were greater than at Palmerston North, and even with the full return of dung and urine from the dry mature sheep used, the soil nutrient cycle was not closed as at Palmerston North. In both trials, the Pedigree New Zealand white clover proved to be a much more effective agent for increasing soil nitrogen than the Pedigree red clover. This, however, needs further investigation as the trials were not designed to test the effectiveness of the associated *Rhizobia,* or of nutrients other than those in lime and superphosphate.

With the forage crops grown subsequent to the pasture treatments, there was a correlation between crop and pasture yields, similar to that secured in the Palmerston North trial. The evidence emphasises the continuing need to increase and conserve soil nutrients, particularly soil nitrogen, in order to obtain the heavy yields of pasture or crop which are possible under New Zealand climatic conditions.

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PASTURE GROWTH AND SOIL FERTILITY

VII. GENERAL DISCUSSION OF THE EXPERIMENTAL RESULTS, AND OF THEIR APPLICATION TO FARMING PRACTICE IN NEW ZEALAND

By P. D. Sears, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

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Summary

The results from the field trials at Palmerston North, Gore, and Lincoln, reported in Parts I—VI, are discussed in relation to the basic concept of pasture growth and soil fertility increase within New Zealand, and to some particular aspects of farming practice.

Introduction

Parts I–VI[1, 2, 3, 4, 5, 6] of this series reported results from field trials at Palmerston North, Gore, and Lincoln, which were designed to measure the effects of white and red clovers, lime and superphosphate, and the grazing animal, on yields and composition of pasture, and on soil fertility as shown by rape and maize crops grown subsequent to the pasture. The data as a whole, fit a pattern of soil fertility increase, through the effective use of clovers, mineral fertiliser, and of animal dung and urine. The stock grazing and treading are additional factors in maintaining the balance between desirable grasses and clovers, and in control of weeds.

It is not the purpose of this paper to discuss the data from the trials in detail, but rather to consider in outline, the main features of this overall pattern of pasture and soil fertility development, and the different effects of the various components of the soil—plant—animal cycle. In addition the application of the results to current New Zealand farming practices is briefly considered.

It is first essential to appreciate the general ecological and economic background to pastoral farming in New Zealand. The main features of this are:—

(1) Over most of the country pasture growth has to be maintained against the threat of invasion by scrub and forest precursors of several kinds[7, 8, 9].

(2) It has been demonstrated that over most of the country the correction of phosphate deficiency is an essential prerequisite for vigorous growth of a balanced pasture[10]. In recent years the application of potassium[11] and molybdenum[12] has been found necessary in some areas. Small dressings of copper and cobalt are also required in certain districts for the maintenance of stock health[13]. The correction of these deficiencies has made possible the grazing of stock and thus the economic development to pasture of large areas which were previously suitable only for forestry[14].

(3) Although there is an extreme paucity of lysimetry data in New Zealand[15], evidence points to considerable losses of soil nitrogen both through leaching and loss to the atmosphere[16]. Nitrogen must therefore be considered a major and continuing soil nutrient deficiency in New Zealand. Synthetic nitrogenous fertilisers which have to be imported are expensive relative to the value of the meat, wool, and milk products which form the bulk of the country's farming output.

(4) The humid temperate climate which promotes succession to rain forest, is also conducive to vigorous growth of herbaceous pasture legumes and grasses. The presumed high losses of nitrogen can be offset by the perennial legumes, white clover, red clover, lucerne, and *Lotus uliginosus,* which are able to survive the annual climatic extremes over most of the country, while for those districts subject to summer drought, the annual clovers can fill an important place. The mildness of the New Zealand winter is a most important factor for continued growth of these annual clovers, after an autumn re-establishment from summer-shed seed. Subterranean clover[17] has proved of particular value in this respect and also serves as a pioneer plant in the development of land that initially would not support the summer growth of the perennial clover species.

(5) In New Zealand, for climatic and economic reasons, animals are continously grazed out of doors. Further, meat and milk production is on a seasonal basis so that at no season is there a great disparity between pasture growth and animal feed requirements. As a result, there is a continuous turnover of soil nutrients by the plant—animal complex without undue interruption of the cycle such as occurs where much of the herbage produced in spring and summer must be conserved for winter feed. This not only avoids the costs and nutrient losses inherent in the pasture conservation process[18] but also makes easier the maintenance of the balances between clover, grass, and soil nutrient supply.

Under these conditions, which are characteristic of New Zealand farming, plant nutrients pass rapidly and frequently from soil to plant to animal to soil and so on. Farm production can be maintained at a high level by the frequent repetition of this fertility cycle. Initially, any deficiencies in soil fertility must be repaired and generally there is a continuing need for some fertilizer to help replace losses from leaching, fixation, or removal in farm products.' But these requirements are often small considering the low reserves in the soil and the high productivity of the land. Other essential requirements for the efficient operation of the fertility cycle are the presence in the pasture of good clovers for the supply of nitrogen, and the control of grazing animals to achieve the best growth and utilization of the herbage, and the even return of dung and urine to the pasture.

When these conditions are fulfilled there is maintained between the pasture animals and soil a state of equilibrium at which soil fertility, pasture production, and the output of animal products are at a high level. If any of the requirements of the fertility cycle is not fulfilled an equilibrium state is soon reached at which

the soil fertility, pasture growth, and animal population are at a lower level. The trials give a measure of the importance of each of the main factors in the fertility cycle.

That this concept is far from new can be seen from the literature cited in the previous papers. Moreover, there are several other approaches for pasture production, with the methods and growth limits depending on the local climatic, edaphic, and economic barriers to the growth of the various pasture species. Included among these factors are: (a) the costs and practicability of methods necessary for the control of the grazing animal, such as fencing and watering; (b) disease and insect pests of both plant and animal; (c) the relative costs of fertilisers, both mineral and nitrogenous; (d) the availability and costs of seed of plants suited to the particular growth conditions; and (e) local requirements for soil erosion prevention and for soil moisture control.

Against this background the cycle of pasture development in New Zealand can be considered.

The All-grass Pasture

Where a sward is composed wholly of grasses, the addition of lime or mineral fertilizer will not significantly raise production for any prolonged period, as growth will be limited primarily by the amounts of nitrogen supplied in the rainfall and of that provided by free-living soil bacteria. The added mineral fertilisers may assist in the action of such bacteria, but the total nitrogen fixed will be small, as it is dependent on the amount of readily decomposable organic matter in the soil[19]. Under mowing, with the removal of herbage, the soil nutrient drain is much more rapid than where the clippings are returned[20], or under grazing with the normal return of dung and urine, but the end-result in each case will be a weak growth of species tolerant of low soil fertility. Because of the low total herbage production, the uptake of minerals is also low, and mineral fertiliser applied to such a pasture will be largely wasted. Artificial nitrogen will usually give a striking growth response, but the costs and compatibility of the large amounts needed to maintain high production will preclude the use of this in practice, except for very special purposes. Such a case[21] is the use of low-growing and mat-forming grasses for playing areas, with the soil nitrogen supplied mainly from fertilisers producing acid soil conditions.

In the present trials there were very rapid and large decreases in pasture production in all the cloverless plots. At Palmerston North, the annual production on the N.R. (No Return of dung and urine) series of this cloverless group soon fell to less than 2,000 lb. D.M. per acre, with the progressive replacement of ryegrass, cocksfoot, and timothy, by browntop and Chewings fescue. The nitrogen content of this herbage also declined very considerably to a mean figure of approximately 2 per cent. The estimated annual nitrogen supply to the grasses from rainfall and free-living bacteria in these plots was only 40 lb. per acre. Both from small-

scale trials of artificial nitrogen within the experimental area, and also from comparisons with other trials on the same soil, this production was only about 15 per cent of that from similar pasture sowings where nitrogen was supplied in adequate amount. There were very similar developments at Gore and Lincoln, but at Lincoln they took a little longer, due to the primary water limitation during the early part of the trial.

In the F. R. (Full Return of dung and urine) series of these cloverless treatments there were very similar trends in production and in botanical and chemical composition, although the process was slower, particularly at Lincoln and Gore where the return of the urine was made throughout on the basis of D. M., rather than on the nitrogen in the herbage as at Palmerston North.

No response was secured at any centre from lime and superphosphate on the cloverless pastures after the establishment stages. The very low yields obtained were from relatively short mowing or grazing heights, and it might be argued that limitation of photosynthesizing surface was a major factor. However, very similar soil nitrogen shortages have been demonstrated in other trials of much taller cloverless pastures. For example, with stands of cocksfoot and timothy used mainly for seed production, and maintained in a relatively tall and dense condition to hold a pure association, very large increases from artificial nitrogen were obtained in herbage and in seed yields [22, 23], although mineral fertilisers alone gave no response. Similar patterns of growth responses have been demonstrated on cloverless hill country pastures given relatively long spells between grazings[24]. A major difficulty with such pastures, even though they are occasionally recommended for prevention of soil erosion, is their unpalatability to stock, and the development of bracken fern and scrub.

The Grass-Clover Association

The effective functioning of the clover plant removed, in varying degrees at the three centres, the nitrogen deficiency of the all-grass association. Its role can best be appreciated by consideration of the hypothetical case where pasture is being established in the absence of the grazing animal on land of low fertility with respect to nitrogen and to one or more essential mineral elements. In this case initial success with a mixture of grass and clover seeds will be assured if the soil nutrient level is raised to the minimum necessary for growth of both leguminous and non-leguminous seedlings. If at this stage the clover seedlings are infected with an effective strain of *Rhizobium*, they automatically become autotrophic for nitrogen, and their subsequent development will be determined by adequacy of essential mineral elements. Fertilizer practice must therefore be directed to this end[25]. (The assumption is made that soil structure defects and gross moisture deficiency or excess are not limiting.)

Within a few weeks of successful infection, part of the nitrogen fixed by the clovers becomes available by underground transport to the grasses growing in association, the time and amount of such transfer being determined by a number of environmental and hereditary

factors[26]. This transfer becomes increasingly important with the passage of time. Provided that sufficient grass plants have survived the early period of low soil nitrogen levels and of shading by the dense clover growth, they will increase in vigour from the increasing soil nitrogen supply, and from their better utilisation of original or applied mineral elements[27].

In the N. R. group of the present trials, the inclusion of pedigree white clover and red clover in the pasture led to markedly higher production. In every trial the yield of pastures comprising both grasses and clovers was several times as great as the yield of pastures of grasses only. It is desirable to examine the effects of red and white clovers separately, and for this purpose results from sub-treatments receiving lime and superphosphate at all centres may be compared. (Although no simple mixture of grass and white clover was included at Palmerston North, red clover virtually disappeared in the last two years of the trial).

For the final year of each trial the yields (Mowing series) of the white clover in the grass + white clover mixture were: at Palmerston North, 6,100 lb.; at Gore, 3,900 lb.; and at Lincoln, 5,500 lb. D. M. per acre. The yields of the associated grasses were 4,600 lb., 3,800 lb. and 1,200 lb. D. M. per acre respectively. The grass yields in the similarly fertilised but cloverless sub-treatments were: at Palmerston North, 1,800 lb.; at Gore, 1,100 lb.; and at Lincoln, 1,100 lb. If the results from all centres are combined, the increase in total production through inclusion of white clover amounts to over 500 per cent, while that of the associated grasses was itself over 100 per cent. In all plots containing white clover where phosphate nutrition was adequate, ryegrass was the dominant grass; in the absence of clover, browntop was dominant. Similarly, there were markedly higher nitrogen levels in the herbage of the grasses associated with white clover than in those growing alone.

On the other hand, red clover was not as effective as white clover in supplying nitrogen to the associated grasses. Grass + red clover associations were included only at Lincoln and Gore, there being no counterpart at Palmerston North. From a consideration of the results of the final year, the high red-clover yields (Gore, 4,800 lb.; Lincoln, 3,700 lb.) did not affect the growth of the associated grasses so markedly, either in yield or botanical composition, as did white. At Gore, the total yield of the grasses growing in association with red clover, was some 1,700 lb., of which 800 lb. was ryegrass, while at Lincoln the grass yield was 900 lb., which was mainly ryegrass. Hence, red clover produced a greater effect on botanical composition than on yield of associated grasses, but by neither criterion was it so effective as white. On the other hand, the summer feed value of the red clover was well demonstrated both in this series and in the previous paddock grazing trial at Lincoln[28].

From chemical analyses of the herbage from Gore it was estimated that the annual nitrogen in the herbage fixed by the white clover was 230 lb. per acre, with a sub-surface transfer to the grasses of 55 lb. per acre. On the other hand, the annual nitrogen fixation by the red clover was 220 lb. per acre, but with a sub-surface transfer to the

grasses of only 23 lb. per acre. At Palmerston North it is estimated that nearly 500 lb. of N. was fixed per acre per annum by white clover, with a sub-surface transfer to the associated grasses of some 140 lb. It is of interest to recall, also, that at Palmerston North the effective nitrogen fixation and sub-surface transfer from poor agronomic strains of white clover was only about one-half that of the pedigree strains used in this trial. These estimates should be compared with those of the amounts annually supplied by free-living soil bacteria, which were, at Gore, 20 lb. per acre, and at Palmerston North, approximately 40 lb. per acre.

Responses to superphosphate were recorded at all centres in the grass + clover N. R. sub-treatments. The actual increases are, however, not of so much interest as are the associated botanical changes. The differences in the extent of the responses were undoubtedly due to the original soil nutrient levels. For example, it appears that at Palmerston North there was no phosphate deficiency at the start of the trial, and that the major value of superphosphate lay in compensating for removal of sulphur. On the other hand, there appears to have been a considerable phosphate deficiency at Gore, while at Lincoln there was also a need for lime.

Despite these initial differences, the changes in botanical composition resulting from the fertiliser application followed the same general trend at the three centres. The initial response to superphosphate was in extra growth of clover, particularly of white clover. This was followed by greater grass growth, mainly of ryegrass. At Lincoln this ryegrass response was very marked in the sub-treatments which also received lime. The most reasonable interpretation of the grass responses is that they followed the improvement in soil nitrogen levels due to the extra clover growth which had been induced by adequate mineral fertilisation.

The effect of fertiliser on the relative growth of the two clovers growing in association was most clearly demonstrated in the Mowing N. R. series at Palmerston North. Superphosphate increased the white clover, but at the same time markedly depressed the red. Whether this depression was due to competition from the white clover, greater disease incidence, or to some other direct damage to the red clover, cannot be estimated from this trial. In the unfertilised mixtures, the red clover was still growing vigorously at the end of the trial, whereas white clover was obviously declining in vigour. Trials have recently been commenced on the same soil to study the effects of the sulphur and phosphorus in superphosphate, under these particular conditions of soil, climate, and defoliation method.

Differences in species composition between plots mown and grazed were observed only at Palmerston North. The major effect of the stock treading and grazing was to keep the sole of the pasture open, and free from low-growing and mat-forming species such as *Poa trivialis,* and to encourage ryegrass. At the same time there was a depression of some of the desired species, particularly of red clover, and to a lesser extent, cocksfoot and even white clover. In general, this comparison between mowing and grazing supported the opinion widely held that stock grazing and treading are factors in maintaining a ryegrass-white clover association at high productivity.

VII.

The Grass-Clover Association with Return of Dung and Urine

The previous section of this discussion considered the growth in a grass + clover mixture under defoliation and complete removal of all the herbage. Under normal grazing the position is, however, very different, as the animal returns at each grazing most of the plant nutrients in the herbage eaten. The amounts and composition of the nutrient return in the dung and urine depend on the growth and production of the animal[29], and also on the herbage composition and digestibility of the nutrients.

The separate and combined effects of dung and urine on pastures were examined in previous trials at Palmerston North[16, 30, 31] and Lincoln[28]. Urine promoted marked local grass dominance within the urine patch, presumably because of the high rate of nitrogen application, while clover was depressed due to competition, and possibly to the toxic effect of certain urinary constituents[32]. Most of the phosphorus and calcium, together with nitrogenous materials of low availability, were excreted in the dung which becomes incorporated into the soil by earthworms. An initial clover response to dung was followed by a grass response.

The present series showed very clearcut growth responses to dung and urine. At all centres dung and urine applied in proportion to herbage production resulted in greater total production, both with and without added fertiliser. The Palmerston North trial furnished the most clearcut example. Here, there were apparently no original soil mineral deficiencies, while the returns were on a more strictly proportional basis than at Lincoln and Gore. In the N. R. grass + clover mixtures at Palmerston North, progressive responses to superphosphate were obtained as a result of the reduction of the original soil nutrients through removal of the herbage. In the F. R. series there was no response to superphosphate, and all the grass + clover mixtures maintained a vigorous production.

The pasture yields and composition of these sub-treatments in the mowing series showed a clear development pattern, and in the final year, yields were as follows:

N.R. (no fertiliser): 8,600 lb. D. M. per acre; ryegrass—8%, other grasses (mainly browntop)—29%, white clover—35%, and red clover—28%.

N.R. (+ superphosphate): 11,000 lb. D. M. per acre; ryegrass—19%, other grasses (mainly cocksfoot)—22%, white clover—56%, and red clover 3%.

F.R. (with and without superphosphate): 14,500 lb. D. M. per acre; ryegrass —54%, other grasses (mainly cocksfoot)—14%, white clover—32%, red clover—nil.

Parallel with the yield increases and the rise in the proportion of ryegrass, resulting from superphosphate, and dung and urine, there was a markedly better spread of seasonal production, a matter of extreme importance in New Zealand where the winter climate permits outdoor grazing. At Palmerston North there was an increase in winter production of over 60 per cent from the superphosphate in the N. R. series, and a further increase of some 50 per cent from the return of dung and urine. At Gore, although winter growth was less than at Palmerston North, the percentage increases in the winter were

greater. Superphosphate gave a 40 per cent increase in the N. R., and 100 per cent in the F. R. series, while the average increase due to the dung and urine was over 70 per cent. Early spring growth was similarly greater in the F. R. series. The Lincoln data were insufficient for such estimates, but in the previous grazing trial[28] the winter and spring growth on the F. R. series was 6 per cent of the annual total, whereas on the N. R. series it was only 1 per cent. This extra winter and spring growth resulted from the dominance of ryegrass which has a higher potentiality for winter growth than the other grasses, and from the greater supply of soil nutrients, particularly nitrogen.

Thus it can be concluded that although the grazing animal does not normally add any plant nutrients, it does play the extremely important role of transforming most of the nitrogen fixed in the clover plant, into a form very suitable for the growth of the associated grasses. Also, by its action in "filtering" out for its own needs the metabolizable energy constituents with a minimum of the nitrogen and minerals in the herbage, and returning *to the pasture* the balance of the latter in its dung and urine, the grazing animal conserves soil fertility to an extent depending on the class and condition of the animal. In so far as the rapid turnover of soil nutrients through the animal stimulates growth of high producing species and the grazing and treading assist in maintaining them against the poorer species, the animal can be said to build soil fertility.

An illustration of this is found in the development of new pasture on land of low natural fertility. In its early stages the new pasture is clover dominant. The animal eats this herbage and the resultant dung and urine swings the pasture balance towards grass dominance. At a later stage the grass constituents will be receiving nitrogen from five main sources: (a) rainfall; (b) the decay in the soil of the free-living soil organisms; (c) underground transfer from clover roots; (d) the soluble N constituents in the urine and dung—these are applied in highly concentrated "doses" at irregular intervals over the paddock; (e) from excretions from earthworms living on dung and dead organic matter, and from other organisms such as grass-grub feeding on live tissue. An interesting conclusion is that the clover, which is the main activating agent, tends to be eliminated from the association both by competition and shading from the grasses, and by direct toxic effects from the urine. The demonstrable fact that clover does not entirely disappear can only be the result of nitrogen losses from the cycle. Alternatively, a pasture deteriorating through lack of soil nutrients, will have the clovers replaced by low-producing and mat-forming grasses, and thus again give a grass dominant pasture, but this time at the low end of the production scale.

An important point to be considered in the interpretation of these trials is that, although the urine was returned for the most part in "doses" as in normal grazing, the return over a period of time became uniform over each plot. At each application the dung was spread evenly. Under these conditions the nitrogen and phosphate cycles appeared to be very efficiently closed at the top production levels at Palmerston North, as was evidenced by the lack of response to added nitrogenous or phosphatic fertilisers on the grass + clover F. R. series at that centre. The effects of soil fertility transference by stock dif-

ferential grazing and excretions between "day" and "night", has been demonstrated in the trials reported in Part V[5]. Many other examples of such transference of fertility, both within paddocks and between paddocks, are readily seen on most farms[29].

Earthworm and Grass-grub Populations

In view of the high correlation between pasture yields and the populations of earthworms and grass-grub, it is important to consider the influence of these organisms.

There is evidence (referred to in Section IV)[4] that earthworms can be a major factor in the improvement of soil structure. Their main food supply appears to be dead plant tissue and dung, and thus, with an increase in pasture growth and stock-carrying capacity, there is a parallel increase in worm population and activity. The important net result is a rapid incorporation of the dung and dead tissue into the soil, with an increase in water-stable soil aggregates and in available plant nutrients.

The earthworm can therefore be regarded as functioning in the soil fertility cycle in a manner complementary to that of the animals grazing and excreting on the surface of the soil, and so the earthworm increase in good pastures must be viewed with favour.

The high correlation between pasture growth and earthworm populations also suggests a useful method of estimating the previous pasture production, at least under the edaphic and climatic conditions obtaining at Palmerston North. Such an estimation method would be of value where direct pasture or stock carrying measurements are impracticable.

A much different outlook should apply with the grass-grub, as it apparently feeds mainly on living roots of the pasture plants. Fortunately, direct chemical control of the grass-grub is now practicable without danger to earthworm populations. Many farmers take action to control grass-grubs only when the pasture has deteriorated to the stage when the turf can be lifted off the ground. The damage is most evident after a period of dry conditions when the shortage of roots is, of course, most important. Some are of the opinion that moderate grass-grub activity may confer some advantage from its soil structure increase, and also from root pruning. Against this argument, however, are the field observations that the grass-grub appears to show preference for the roots of desired species (particularly short rotation ryegrass and white clover) over those of other species e.g., cocksfoot. Thus, although the damage may not be drastic enough to excite immediate attention, pasture deterioration progresses. It appears to the writer that, on balance, the evidence is strong for continued action against the grass-grub. This applies particularly with pastures of high productivity containing short rotation ryegrass, and to those areas subject to soil-moisture deficiency.

Development of Soil Fertility

From the soil analyses at Palmerston North, and from the yields and composition of the forage crops grown at all centres subsequent

to the pasture treatments, it is apparent that the residual soil fertility paralleled the previous pasture productivity. Emphasis has been placed throughout the trial on soil nitrogen, but there were also large differences in content of soil organic matter and mineral constituents. These all undoubtedly contributed to the large fertility differences as measured by appearance, yield, and chemical composition of the subsequent crops of rape and maize. At Gore, rape crop yields ranged from 4,300 lb. to 10,200 lb. D. M. per acre, and at Lincoln, although all yields were probably reduced by dry conditions, from 2,900 lb. and 5,200 lb. D. M. per acre. At Palmerston North, after the first ploughing of the grazing series, the first growth of the rape crop on the fertilised cloverless N. R. treatment was 8 in. high and had leaves of yellowish-green, with much purple coloration on the leaf margins. With the recovery growth, it gave a total yield of 11 tons of green matter (4,300 lb. D. M.) containing 57 lb. N, 14 lb. P, 112 lb. K per acre. At the other extreme, the crop after the fertilised grass + clover F. R. treatment was 30 in. high, had dark bluish-green leaves with only slight red-purple leaf marginal colour, and gave a final yield of 39 tons of green matter (10,900 lb. D. M.) containing 234 lb. N, 42 lb. P, and 361 lb. K per acre. Similarly, with the maize crops at Palmerston North there was a fourfold difference in yields, and a large difference in N. percentage between these treatments.

The implications of these results are of great economic importance. For example, from these data it is reasonable to conclude that for leafy forage crops on land ploughed out of run-out (i.e., cloverless and weedy) pastures, it would be necessary to apply some 4-6 cwt. of sulphate of ammonia (or its equivalent) per acre to get a yield equal to that which would be secured on land ploughed out of highly productive pastures. This assumes that the applied nitrogen is completely used and that other nutrients are not limiting. This last assumption is, of course, far from justifiable, as pastures may be poor because of soil mineral deficiencies. It is therefore likely that other fertilisers may also be needed for the production of a heavy leafy forage crop after poor pasture. On the other hand, although it is highly probable that good yields of leafy forage crops will be secured without any fertiliser, on land ploughed out of highly productive pasture, there may still be some nutrient deficiency or imbalance in the soil which may not have affected the pasture growth, but which is of real importance to the forage crop. An example of this was seen in the limed grass + clover treatments in the grazing N. R. series at Palmerston North, where the rape yields were lower than in the unlimed treatments. There were indications that lime had induced a magnesium deficiency. In this case a deficiency which did not influence pasture growth showed up in the subsequent crop.

The general conclusion must be that the best preparation for a heavy crop is a highly productive pasture, and that after poor pastures, the crops will also be poor, unless nitrogenous and probably mineral fertiliser are applied in generous quantities.

The other outstanding feature was the marked rapidity with which the induced soil fertility differences disappeared. At all centres there was virtually no difference between sub-treatments in the growth of the crops after the first rape crop. These crops were, however, all

cut and removed. This would naturally result in a more rapid soil exhaustion than under grazing with the normal return of the dung and urine.

Considered in relation to the cycles of soil fertility under discussion, the non-leguminous forage or grain crop simply replaces the grass element in the pasture. A grassy silage, hay, dried grass, or seed crop will deplete the soil in the same way. Soil nutrient depletion by the crop will depend on (a) the species, (b) climatic conditions, (c) weed competition, and (d) the utilisation or harvesting system. In addition to the nutrients actually removed in the crop, the total effect on soil fertility will include the losses from the soil during the cultivation and growth period.

The cropping phase can usefully be considered as a culminating point in any cycle of soil fertility, the yields secured being a good index of the effectiveness of the previous pasture treatment. The reduction in soil nitrogen levels by cropping can also be useful in assisting clover establishment in the subsequent new pasture[33].

SOME PRACTICAL APPLICATIONS OF THE RESULTS

The results provide quantitative data indicating the requirements for "good pasture management" under average New Zealand farming conditions.

A generalised systematic application of the results to the improvement of New Zealand farming practice can be made by a separate consideration of (a) use of the best species, (b) dung and urine return, (c) mineral fertiliser requirements, (d) grazing control for the best balance between grasses and clovers, (e) cropping rotations and utilisation practice, (f) control of insect pests. It is very obvious, however, that there is a large degree of overlapping of these factors; the true art of grassland husbandry consists of balancing them to best advantage under the existing set of physical and economic combinations of possibilities on any particular farm. Examples of the use of the general background provided by the data from these trials, in discussing particular grassland problems in this country, have been given in recent papers by the author[34, 35, 36].

The following is an attempt to recapitulate some points of major importance. A summary of grazing control on established pastures is not, however, separately considered. This is because of the many complexities, due to regional and species differences in seasonal and total growth, palatability, and feed value under different defoliation heights and intensities, and to differences in feed requirements and utilisation between the grazing animals[37, 38].

USE OF CORRECT SPECIES AND STRAINS

The greatest production increase in the trials was that resulting from the inclusion of clover, the extra production being due to both clover growth and enhanced grass growth. In the main experiment the pedigree white clover had a greater influence on grass growth than did red clover, but the latter also served a useful purpose, particularly by its summer and autumn production.

In most parts of New Zealand the incorporation of pedigree white clover into the pastures is the essential starting point of pasture improvement. Under conditions where white clover is unable to persist through the summer, the deeper rooted red clover or lucerne, or the annual subterranean clover, can be used to advantage, either by itself, or to supplement the white clover until this species becomes the dominant legume. *Lotus uliginosus* is of value for shady wet hill faces, or on swampy land where fertiliser and full animal grazing or mechanical weed control is impracticable. Strawberry clover shows to advantage on coastal saline flats subject to periodic inundation.

To take advantage of increasing fertility through these legumes there is the parallel need for grasses of better persistence, total and seasonal production, palatability, and disease resistance. The list of pedigree strains of grasses now available in commerce from the plant breeding programme at this Division[39], includes Italian, perennial, and short rotation ryegrass, cocksfoot, and timothy. This range can fulfill requirements for pasture improvement in New Zealand, although *Paspalum dilatatum* is an additional valuable plant in northern areas, particularly for semi-swamp areas.

Many other species are present in most pastures. Both on the flats and the hills, Yorkshire fog, browntop, sweet vernal, and danthonia undoubtedly contribute a large proportion of the grass production at the present time[40, 41, 42]. Improvement programmes aim first to increase the production from such species, and finally to replace them progressively by higher production species. It is, of course, essential that soil fertility be increased to the level where these better grasses are not limited for soil nutrients.

The introduction of the better clovers and grasses can be made by oversowing on to the established pasture, or after full cultivation. Although procedural details are different for these two methods, the general principle to be followed is the same. The seedling plants have to be protected against excessive competition and against excessive damage from the grazing animal. Thus, for introduction of clovers by oversowing into established pasture, heavy grazing before sowing (to open up the sole of the existing sward), and continued grazing after the new seedlings have struck (to prevent overshading from the growth of the existing plants), have given much better results than where tall growth is allowed[25]. Similarly, with completely new sowings attention should be paid to: (a) seeds mixture and sowing methods[8, 25, 43]; (b) pasture height at and intensity of early grazing[44, 45]; (c) weed populations[35, 40]; (d) soil nitrogen levels at time of sowing[33]; and (e) the soil moisture levels and liability to poaching of the land during early grazings.

Effective *Rhizobium* infection is of course a key factor for success. In this series of investigations, inoculation of the clovers was not carried out and reliance was placed on infection of the clover plants by strains of *Rhizobium* existing in the soil. This is the universal practice in New Zealand. However, during recent work[25, 47], on the establishment of clovers on pumice land which is being directly converted from native scrub to pasture, striking responses from inoculation with good *Rhizobium* strains have been obtained. The whole question of natural *Rhizobium* populations and their effectiveness throughout the country is due for critical re-examination.

VII.

Dung and Urine Return

The methods whereby plant nutrient elements are transferred through the dung and urine of the grazing animal have been described in this series and in previous papers[29, 48, 49]. The effects of "day" and "night" grazing, of permanent hay and silage areas, of water troughs, gateways, shelter belts, and of any other factor which affects the even and complete return of the dung and urine to the pasture which produced it, all lead to transfer of fertility, and are all under the farmer's control to a greater or less degree.

The even return of excreta within each paddock and within the farm is basically desirable, but cases can exist where fertility transfer serves a useful purpose. For example, areas can be rapidly improved (e.g., for cropping) by night grazing, and, of course, by the manure collected from cow and pig yards[50]. At the other extreme, grazing during the day only, or by large numbers of hungry stock for periods of only an hour or so, can be used to decrease fertility, e.g., for better clover establishment in new pastures where the early grass growth is very vigorous.

Hill country presents special problems of fertility transference due to the habits of the grazing sheep and cattle in making tracks along the contours, and in "camping" on the high land at night. This leads to fertility transfer from the "riser" areas to the tracks, and from the slopes to the tops[29]. Added to this fertility movement there are undoubtedly large differences of soil temperature and moisture between "riser" and step areas resulting from the differences in slope.

There is some indication in recent work, however, that a better spread of animal manure follows the increased stock carrying capacity induced by the introduction of good clovers and their fertilisation with superphosphate[24]. Although controlled experiments were not conducted, a conscious effort was made in this investigation to apply the fertiliser preferentially to the "risers." This was done to encourage vigorous and palatable growth on those areas which are continually being depleted by the grazing animals.

Mineral Fertilisers

Before a mineral fertiliser will give a response three prerequisites have to be fulfilled. Firstly, it must supply an element or elements which are limiting growth, secondly, no other element shall be limiting, and thirdly, other conditions for growth must be satisfactory. In view of the quantitative importance of nitrogen in pasture plant nutrition, it is not surprising that no responses to superphosphate and lime were obtained when clovers were absent. It follows that the nutrition of the clover plant, particularly at the early stages of land development, is of prime importance. It is equally important to appreciate the reasons for the lack of response to these minerals at high pasture production levels.

In practice the changing needs of the soil for any particular mineral element must be provided for by adjustment in fertiliser practice. In the present trials only calcium, phosphorus and sulphur were applied;

if the trials were repeated in other areas, or had been continued longer on the same areas, other essential elements would no doubt also have required attention.

The above attitude is very different from that of the very large majority of New Zealand farmers who each year apply a uniform amount of phosphatic fertiliser (normally superphosphate[10]) over the whole farm, without any appreciation of natural or induced differences in soil nutrient requirements within the farm, or to botanical composition of the pastures.

For the determination of mineral deficiencies, the use of plots under the N. R. system on an appropriate grass + clover mixture, or on a pure grass association with added artificial nitrogen, is the most stringent test, and is thus to be preferred for exploratory field trials. The self-contained group of paddocks under grazing is, however, the obvious requirement for providing long-term answers.

It is obvious from the arguments developed in the previous sections that at moderate-to-high pasture production levels, differential fertilisation practice on any particular farm will be determined by stock movement as well as by original soil nutrient levels.

Cropping Rotations

Many New Zealand farmers appreciate the need for high soil fertility to obtain good forage or grain crops, and utilise the value of good pastures to this end[51, 52]. The average crop does not, however, reflect any widespread application of such methods. This appears to be particularly so in the North Island where crops are normally taken only as a subsidiary part of a pasture renewal programme. When the state of the average ploughed-up pasture is realised, the reasons for the generally low crop yields can readily be appreciated. It is also easy to understand that the lack of enthusiasm that many North Island farmers have for cropping, has resulted in most cases from a succession of poor crop yields.

The results from this series of trials give a clear explanation of some of the reasons for crop yield differences, and offer useful suggestions for farm practice in New Zealand. Where pasture has seriously deteriorated before being ploughed up, the taking of a summer or winter crop before sowing down again is of doubtful advantage. Only in exceptional circumstances, or with expensive fertiliser applications, will a satisfactory yield be obtained, and emphasis should be laid on increasing soil fertility as rapidly as possible. This will be achieved better by a programme of sowing direct to grass, with every effort being directed to growing a highly productive pasture which will automatically increase soil fertility.

Conversely, the cashing-in on fertility built up by highly productive pasture is of great importance in achieving maximum production from New Zealand's arable land. Our ability to produce animal products cheaply and efficiently through pasture should not blind us to the other demands which are made on our ploughable soils. For example, New Zealand's wheat harvest for many years has been only a fraction of her requirements, while the potato crop has been insufficient to meet internal demand in two successive seasons. Human food crops can

form an effective counterpart to high production grassland, and the combination represents the most effective use of ploughable land. Not only is the farmer capitalising on fertility built-up through his pastures, but the production of a white crop brings such soil into good condition for sowing down new pastures. Many other factors, of course, enter into such a scheme of land use: the economics of white crop production, incidence of disease, labour supply, and degree of subdivision, are all of great importance. But none of these over-rides the general conclusions reached above.

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MOLE DRAINAGE INVESTIGATIONS IN NEW ZEALAND

II. REPORT ON INVESTIGATIONS OF SOME METHODS OF CONNECTING MINOR MOLE DRAINS TO MAJOR MOLE DRAINS

By H. G. Hopewell*, Massey Agricultural College, Palmerston North

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Summary

Experiments to determine the relative effectiveness of four different methods of connecting minor mole drains to major, or collecting, mole drains, are described.

The "McLeod" method is shown to be the most satisfactory for general use if properly carried out.

The other methods, while requiring little or no labour to effect the junction, are shown to be unreliable under certain conditions.

Of these the "spearing" technique can, however, be regarded as satisfactory in practice, provided ample majors are installed and the falls are good. This method is likely to find favour with contractors.

Introduction

It is common practice in mole drainage operations to collect a number of minor, or lateral, mole drains into a major, or collecting, mole drain. In this way a multiplicity of outlets is avoided and maintenance very greatly reduced. Normally, several majors are connected directly to a short length of tile drain, the outlet to which can be protected and rendered secure.

Various methods of connecting the minors to majors are in use but none appear to have been the subject of detailed study under field conditions.

Hudson and Hopewell[1, 2] studied the effect on the mole channels when one mole drain was drawn across another at various distances above and below it, and at various angles to it. In the light of this work they were able to appraise, to some extent, the relative merits of several methods of joining minors to majors.

The object of the trials described in this paper was to study four of the more promising methods under field conditions.

The methods studied were:

(1) The "30°–40°" method wherein the major drain is pulled away from the outlet at the same depth as the minors and in such a direction as to cross them at an angle of between 30° and 40° (see Fig. 1).

(2) The "McLeod" method, which involves pulling the major across the minors at the same depth and approximately at right angles to them. The passage of the major causes the minor to be blocked and the obstruction is cleared with an auger from a hole dug astride the minor just clear of, and on the lower side of, the major.

* Drainage Research Officer. This work was carried out at Massey Agricultural College, under the direction of Professor A. W. Hudson, with the aid of a grant from the Department of Scientific and Industrial Research. This is the second of a series of papers, the first of which, by Hudson and Fife[3], appeared in 1940.

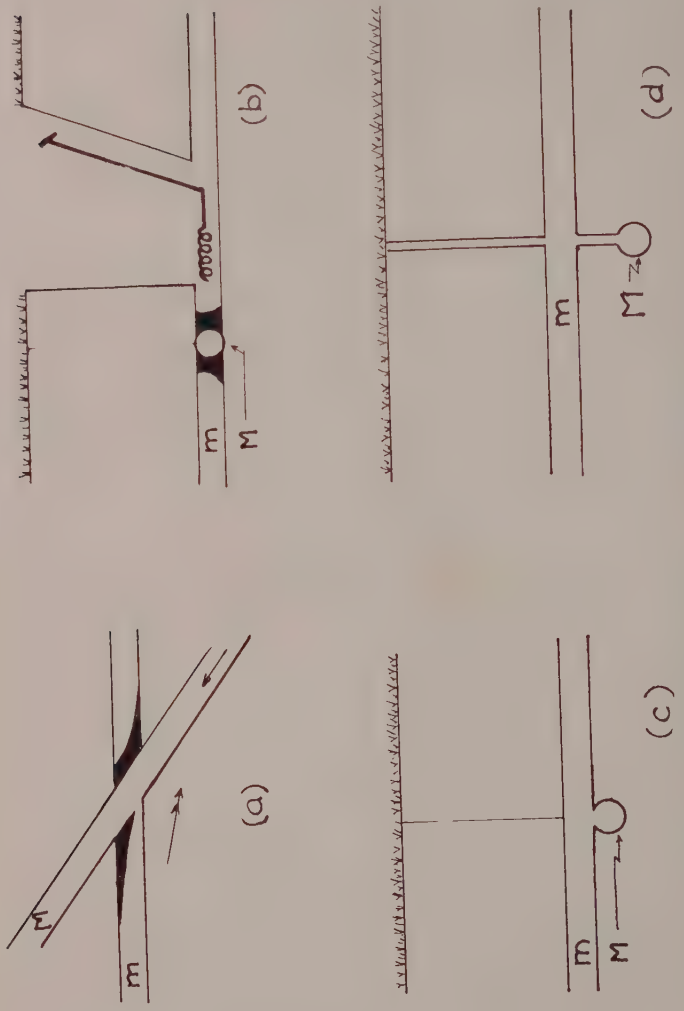

FIG. 1.—Methods of connecting minor and major mole drains:
(a) 30°–40° method. Showing nature of blockage in minor channel (m) caused by passage of major (M). Since this is incomplete on the upper side of the major, water can pass from minor to major. Double headed arrow indicates direction of fall, single headed arrow direction of pull.
(b) McLeod method; (c) Cut-top method; (d) Spearing method.

(3) The "Cut-top" method. In this the procedure is to pull the majors first at such a depth that the floor of the minor drains when subsequently pulled will just cut the roof of the majors.

(4) The "Spearing" method. This involves pulling the majors first a depth 5–7 in. greater than that at which the minors will be drawn. A spear is forced down where the two drains cross, thus providing for movement of water from the shallow minor to deep major.

Soils

The soils of the experimental area at Massey Agricultural College have been described by Hudson and Fife[3], and Fife[4].

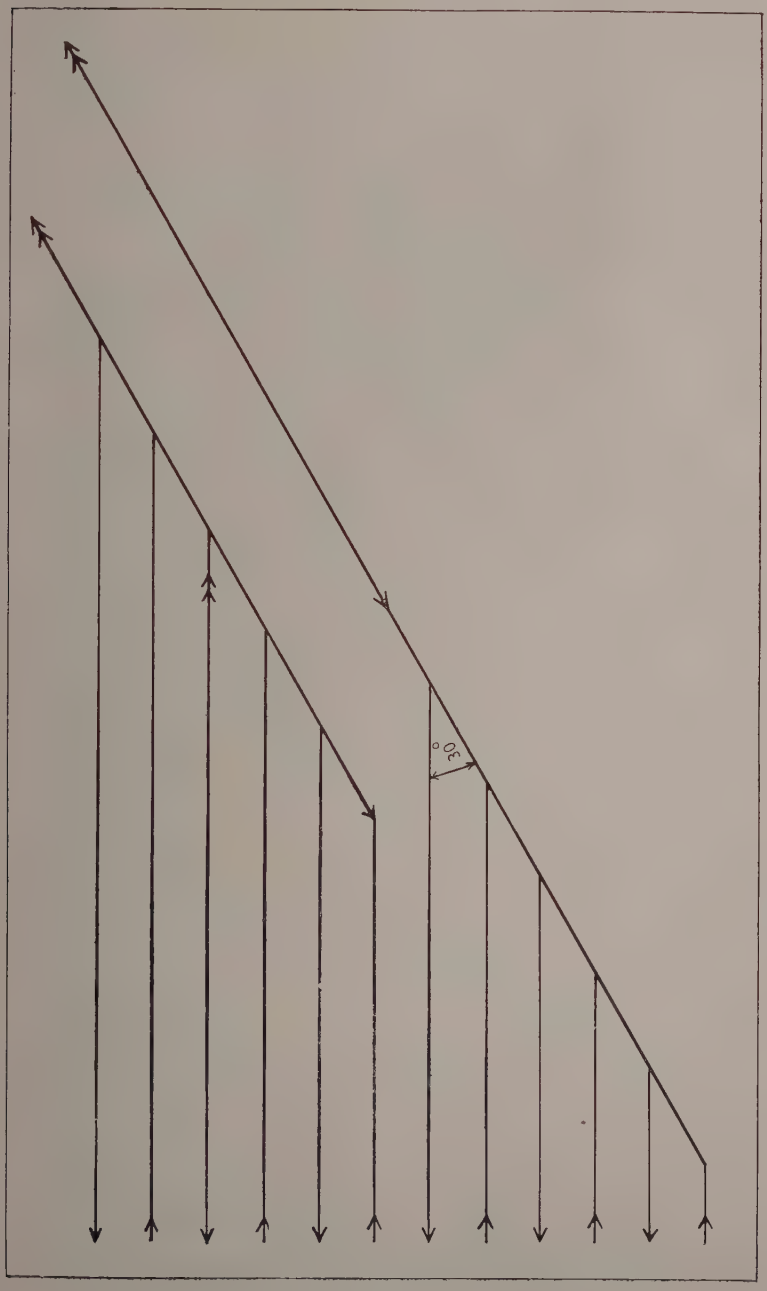

FIG. 2.—Layout of drains in Trial 1. Single-headed arrows indicate direction of pull and double-headed the direction of flow of water.

Experimental Procedure

Trial 1: *Investigation of the Efficacy of the* 30°–40° *Junction*

A series of minor moles each approximately 6 ch. long were pulled at a depth of 20 in., alternatively up and down the slope. They were placed 6 ft. apart at the upper end of the field and converged to 3 ft. apart at the lower end.

Six drains were pulled with the standard equipment with trailing 3 in. plug and six with a combined-torpedo-plug modification described by Hudson and Hopewell[1, 2].

The drains were drawn in November, 1939. Soil conditions were well suited to mole drainage operations. The surface was firm and the subsoil contained only sufficient moisture to make it plastic. The fall was about 1 : 60 near the top end of the field, increasing to about 1 : 30 at the bottom end.

Two major drains were then pulled in the appropriate manner, one across each series of minors. The same equipment was used to pull the major as was used to form the minors which it crossed. (See Fig.2).

The position of each minor was marked about three feet on the upper side of the junction by setting a brick squarely over the minor and flush with the surface of the ground. Testing was done by pouring water into the minor and noting the time taken for it to reach the outlet.

In June, 1940, the top junction of each series was excavated as part of a demonstration to farmers and each was found to be satisfactory.

Trial 2: *Comparison of Three Methods of Connecting Minor to Major Mole Drains*

The methods compared have been described and were as follows:

(1) The "McLeod" method;

(2) The "Cut-top" method;

(3) The "Spearing" method.

The routine mole draining of a field on the College farm was slightly modified for the purposes of this experiment.

The draining was done in July, 1944, a standard light mole-drain plough with 3-in. plug being used.

The ground surface was firm but moist. The subsoil was found to be wetted unevenly at the depth at which it was desired to pull the deep majors for the "speared" series, the depths of which consequently varied somewhat, tending to be shallower where the soil was not wetted to the depth of the drains. These were pulled first at a depth varying between 22 and 25 in. and those for the "Cut-top" series next at a depth of 19 to 22 in. The

minors were then pulled at 18 to 19 in. followed by the McLeod majors at the same depth.

The "speared" and "McLeod" junctions were then completed.

The spearing of the junctions was carried out in two stages. First, a spear with an enlarged end $\frac{9}{16}$ in. diameter was used as a "feeler". This was followed by another spear with an enlarged end $\frac{3}{4}$ in. diameter.

Each minor drain was marked and the junctions tested as described in Trial 1.

The first flows from the drains occurred in September, 1944, two months after pulling.

RESULTS

Trial 1

The junctions were tested in 1940, 1942, 1943, and 1948.

On each occasion water was found to move from each minor to the major. One junction in the series pulled with the combined-torpedo-plug appeared to be restricting the flow of water at each test, and in the final test one junction in the other series was in the same category.

Trial 2

The junctions were tested in 1944, 1945 and 1948 and the results of the tests are summarised in Table I.

(1) "McLeod" junctions: At the first test, 5 per cent of the junctions did not function while 1 per cent were not entirely satisfactory. By the test in the following year, only 1 per cent did not function, 4 per cent having cleared themselves, while 2 per cent gave slow flows suggesting they were partly blocked. The flow from 11 per cent appeared from an outlet further down the field but each junction permitted the flow of water. Presumably the flow was diverted through a drain comprising part of a series installed previously.

(2) "Speared" junctions: At the first test 9.5 per cent of the junctions were blocked and the information concerning 0.5 per cent was doubtful. By the following year, 3 per cent had cleared themselves and 1 per cent appeared partly blocked, 6.5 per cent were ineffective, and the information concerning 8.5 per cent was doubtful because there was some collapse at the top end of two of the four majors involved.

Four years after the junctions were formed 4.5 per cent were definitely ineffective and the information regarding 33.5 per cent was doubtful due to the complication of the progressive breakdown of the top end of each of the four majors concerned. These majors are on flattish ground (a fall of approximately 1 : 100) but indications point strongly to the fact that this type of junction is conducive to silting and breakdown of the major, as reported by Davies[5].

TABLE I. SUMMARY OF RESULTS OF TESTING JUNCTIONS

(Expressed as percentages of junctions under test)

Year	Ineffective	Doubtful	Unsatisfactory	Effective
"McLEOD"				
1944	5	—	1	94
1945	1	—	2	97
1948	3	—	3	94
"SPEARED"				
1944	9.5	0.5	—	90
1945	6.5	8.5	1	85
1948	4.5	33.5	—	62
"CUT-TOP"				
1944	9	—	2.5	88.5
1945	12.5	—	0.5	87
1948	12.5	12	6.5	69

(3) "Cut-top" Junctions: The test in the year the drains were pulled revealed that 9 per cent of the junctions were ineffective and a further 2.5 per cent were unsatisfactory. In the case of one junction, the minor was found to be practically the same depth as the major, which was effectively blocked. This junction was completed by an alternative method. In another instance, a major was found blocked and a check of levels revealed that it had lost fall over a short distance. This probably accounted for the collapse, although the method of forming the junctions may have been a contributing factor. All junctions above this point were ignored in the examination of results. Four years after installation only 69 per cent of the junctions were effective.

DISCUSSION

The results of Trial 1 indicate that the method can be regarded as satisfactory when carried out under soil conditions similar to those obtaining when this experiment was laid down, i.e., when subsoil is moist.

Demonstrations of this method have been made before groups of farmers on numerous occasions and shown to be successful in the great majority of cases. It was observed that on the very few occasions when the junctions did not prove entirely satisfactory, the subsoil contained excess moisture. This suggests that under these conditions the blockage tended to seal effectively, while under drier soil conditions it did not. Whether the shrinking of the clay during the summer's drying-out would provide a pas-

very much greater above the drains. An example of this was seen in the course of recent drainage operations near Palmerston North. At the time the trenches were being dug, a pick was necessary to excavate the subsoil. The material above existing tiles could, however, be removed easily with a spade. The original system was installed 46 years ago.

Nicholson's experiments[2] in connection with mole drains indicate that the process is essentially similar to that postulated by Flodkvist. He states: "The water moves in this (surface) layer in the direction of the surface slope and so arrives at the mole slit and the fissures surrounding it, through which it obtains access to the mole channels".

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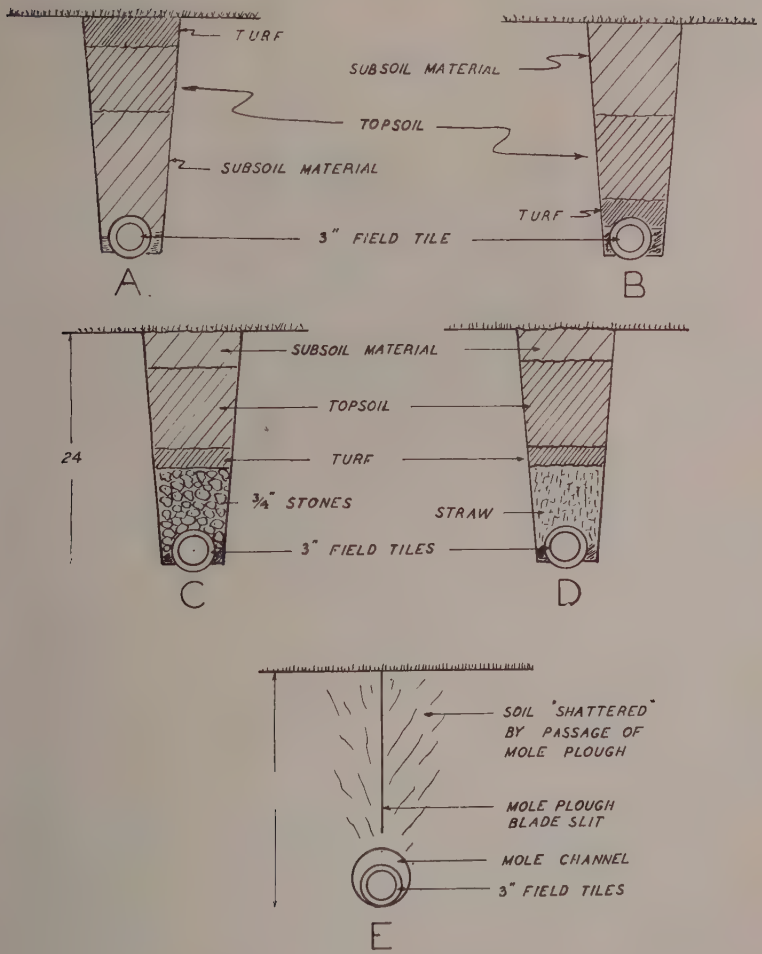
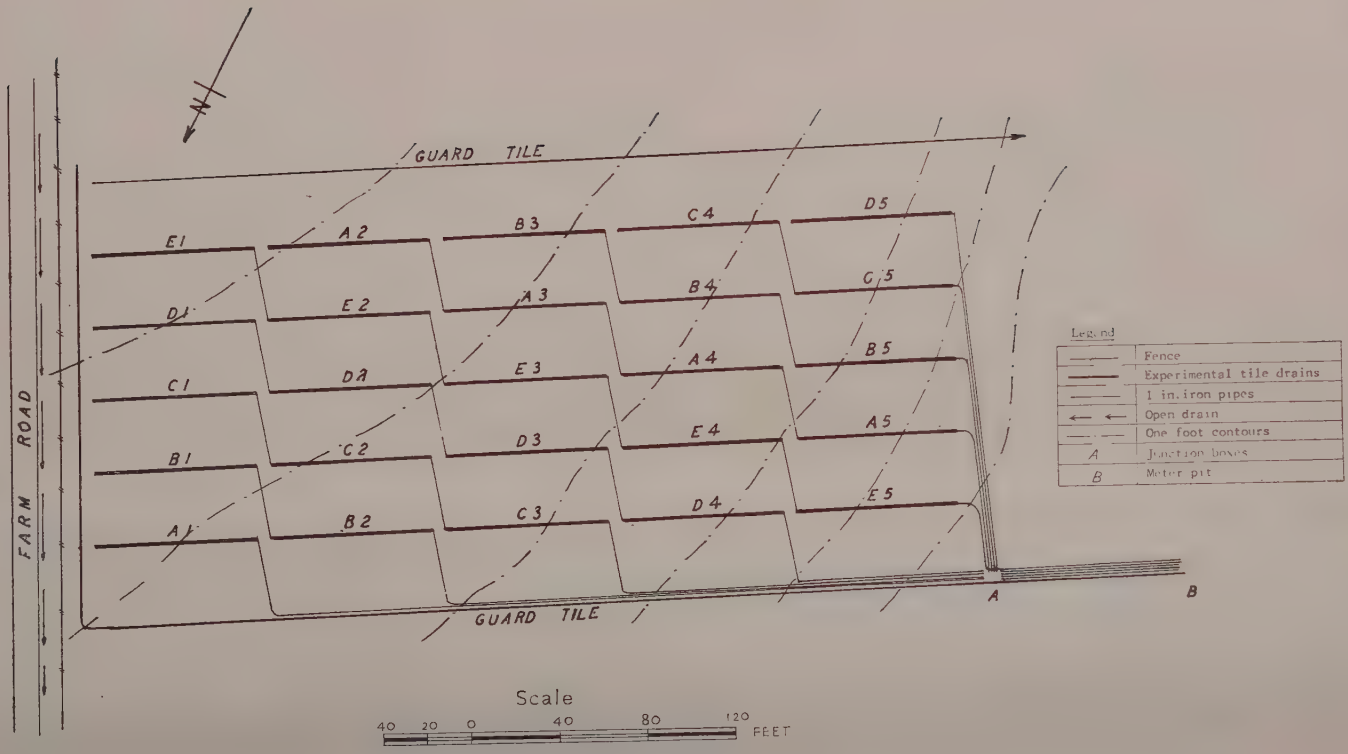


FIG. 2.—Details of treatments

Each treatment consisted of five sections of drain 74 ft. long. Within the limits dictated by practical considerations, the units of each treatment were distributed over the experimental area. Reference to Fig. 3 will show that the sections of Treatment E run diagonally through the area. All other treatments have a section or sections North and also South of these. The sections of any treatment in the Northern part of the area will be seen to lead from one to another and finally down adjacent to the "guard tile" to a junction box situated in a pit at point A. The flow from the sections of any treatment situated in the Southern part of the area is collected and led to the junction box in a similar manner. The combined flow from the five sections of each treatment is then conducted by a single pipe to an enclosure B on the side of the gully where the flow meters are installed.



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The drains were installed in July–August, 1940. All trenches were dug by hand to the same dimensions, being 10 in. wide at the top and 6 in. at the bottom. They were on an average 24 in. deep, carefully graded, and a groove was provided in the floor as a bed for the tiles. The falls varied from 1 in 280 at the upper end of the area to 1 in 50 at the lower. Three-inch earthenware field tiles of good quality were used. These were laid so that they abutted closely at the top, the gaps, if any, between successive tiles, being at the sides or bottom.

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Due to the rapid and marked fluctuations that occur in drain outflows from this type of soil it is essential that automatic continuously recording meters should be used.

A flow-meter devised by Childs[1] was modified by the N.Z. Department of Scientific and Industrial Research. This proved suitable, though some further modifications were found necessary. The Childs meter was fitted with a weir plate provided with holes drilled in such a pattern as to give a linear relationship between height of rise of water on the weir plate and the magnitude of the

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outflow. This greatly simplified the calculation of total and peak flows. It was found, however, that on occasions the holes became blocked, leading to obvious errors.

This plate was then replaced by a weir plate similar to that used by Janert[8] in which the weir notch was so designed that it gave the linear relationship mentioned above. (See Fig. 4.)

The meter is found to give satisfactory records of total flow except during periods of prolonged small flows when the difficulty of setting the instrument accurately at zero leads to some errors.

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Continuous records of outflow have been obtained from 1943 to 1948. From 1940 to 1943 and during 1949, some measurements were obtained with a bucket and stopwatch. In 1950, a limited number of records were obtained. Records from 1943 to 1948 are not complete, due to the fact that from time to time some of the meters have failed to give satisfactory service. They were modified subsequently.

Results based on an analysis of total and peak flows recorded over a ten-year period are presented.

In considering the outflows, particular attention has been paid to peak or maximum flows which it is believed will give the most satisfactory guide to whether or not the nature of the backfill is a factor limiting the movement of water to drains. Fortunately, records of peak flows are more accurate and complete than those pertaining to total flows.

Examination of peak flows show there are no marked and consistent differences between the various methods of refilling the trench (Treatments A–D.) The mole-tile drain (Treatment E) has given consistently smaller flows than the others. However, this treatment has, on occasions, given the greatest flow.

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It is found, however, that the order of efficiency varies from year to year and from one drain performance to another in the same season. It is also a fact that during one drain performance comprising a number of peaks of much the same magnitude, a particular treatment may give a greater maximum flow than the others at one stage and poorer at another.

In general, it appears that at the higher outflow levels, Treatment D (straw over tiles) gives greatest flows, and this would suggest that the material over the tiles in Treatments A, B and C

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Range (gal./ac./hr.)	No. of Flows in Range	Average of Flows Involved Treatment D (straw) (gal./ac./hr.)	Average Peak Flows Relative to Treatment D = 100				
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Examination of total flows show a similar lack of consistency, but in general these are related to the magnitude of the corresponding peak flows.

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It might be expected that certain treatments would commence drainage activity sooner than others after the onset of rain. The records have been examined from this point of view. It is found, however, that the time of commencement of flows cannot be determined accurately due to several factors: (1) the small time scale on the chart (one hour is represented by 0.09 in.); (2) the amount of movement of pens in their guides; and (3) slight irregularities in the timing of the clocks.

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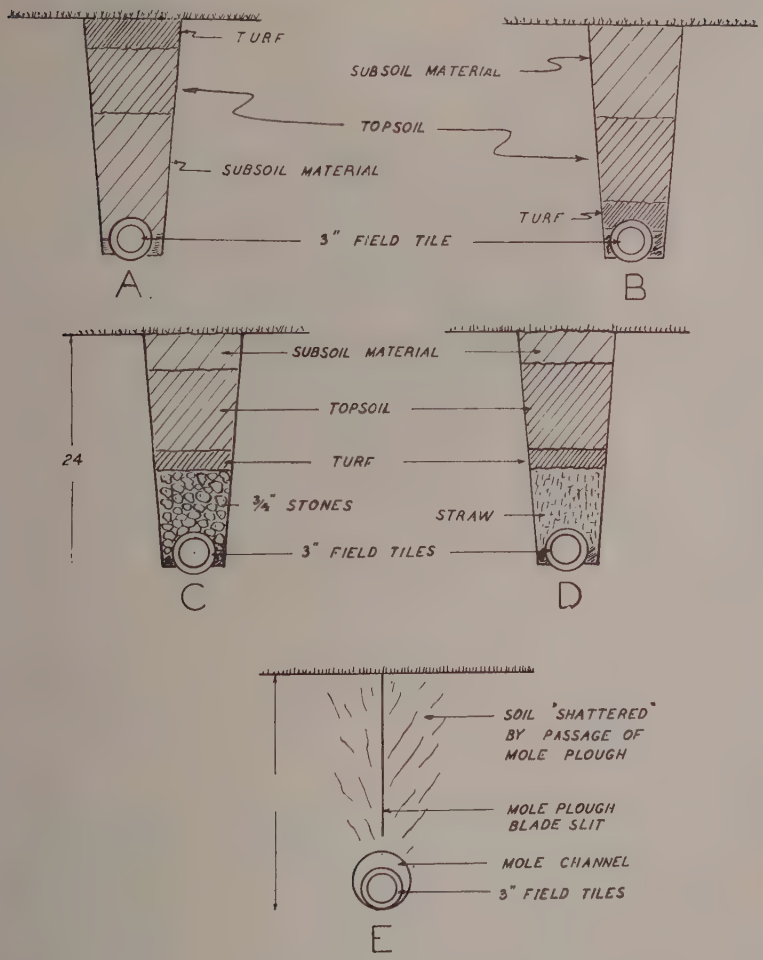

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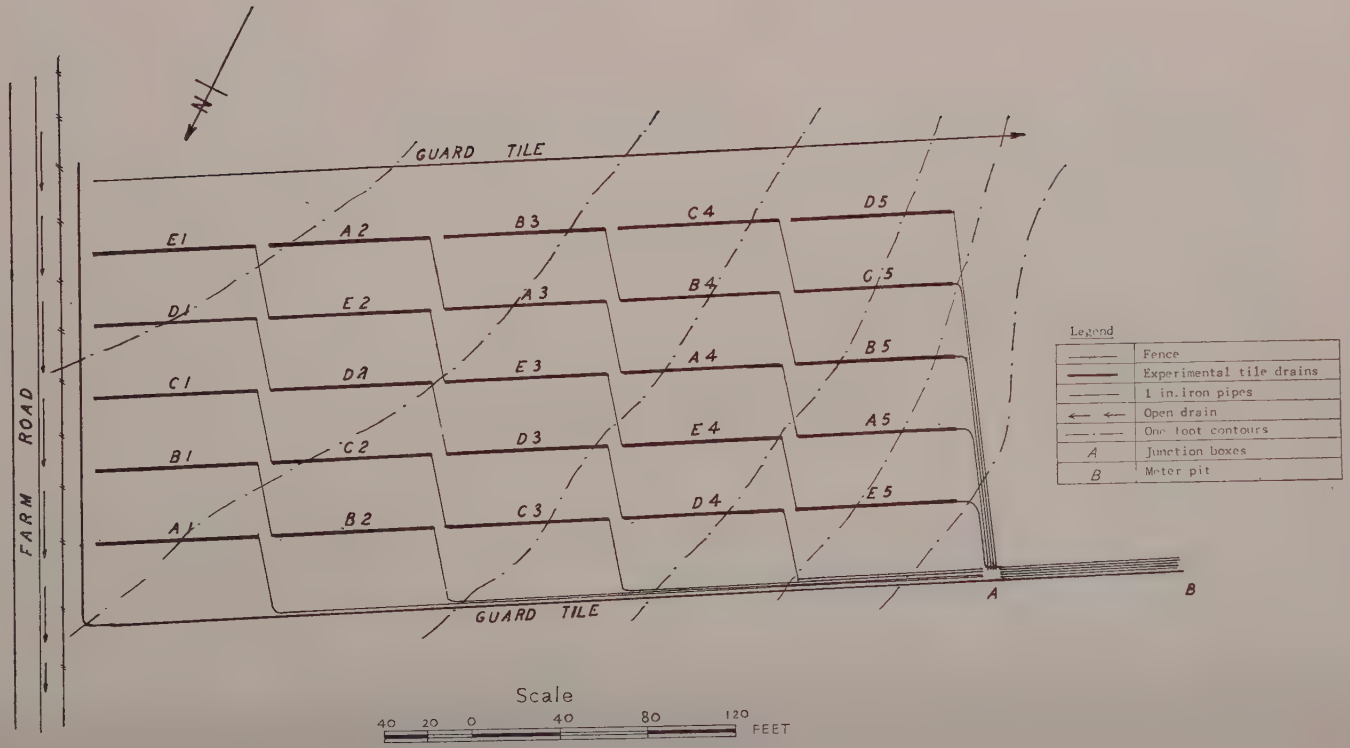

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Before discussing the results it would be appropriate to consider the usefulness of outflow measurements as a means of determining the relative efficiency of various drainage practices.

The value of this technique depends on the premise that the drains which dispose of the excess water most rapidly and in greatest quantity will produce soil conditions most favourable to the growth of normal crops and pastures.

This, it is claimed, can be assumed to be the case, at least, where drains at the same depth and distance apart, but varying in some other respect, are being compared. It is therefore believed to provide a reliable guide in this experiment.

The author, however, wishes to draw attention to one particular set of circumstances under which the validity of the premise would be open to question. On occasions, during the summer and early autumn, intense rains have caused small flows from drains even though the soil was not wetted below an inch or two. One instance has been recorded where an appreciable flow occurred on an area adjacent to this experiment. In this case mole drains were installed in December and by March the slits associated with the drains had opened, due to the dry conditions prevailing. Consequently water was able to move freely down to the mole channels.

The autumn growth on the drained area was observed to be less than on another part of the area which was undrained but otherwise similar. It was concluded that the undrained ground retained a greater proportion of the rainfall and that this accounted for the improved growth.

It is considered, however, that such an effect would be important only in the first or second summer following the installation of tile or mole drains.

Another drawback with this technique is that no information is available which will enable drain performance and crop or pasture production to be correlated. For this reason the results obtained can be interpreted in terms of effect on plant growth only in a general way. However, since it is very probable that small differences in total and peak flows would have no measureable effect on crop yield, the technique may well provide a more critical means of determining the relative efficiency of certain drainage practices than would the measurement of production from experimental drainage plots.

DISCUSSION OF RESULTS

In the main, Treatment D (straw over tiles) has given most intense flushes. The possible importance of this aspect, however, should be considered in relation to the fact that only three or four flushes of this intensity occur in a normal season and these are of short duration.

Furthermore, four of the sections of this treatment are adjacent to and on the lower side of the mole-tile drains which are generally disposing of less water than the other treatments. The possibility that water that would normally be removed by these less effective drains might move to the adjacent drains cannot be overlooked. This reveals a weakness in the experimental design and in sub-

sequent drainage experiments provision has been made to guard against this eventuality.

The smaller outflows from Treatment E (mole-tile drain) indicate that the water has not been able to enter drains as rapidly as it does where tiles were installed in the conventional manner.

The difficulty of deciding whether the differences observed would be of importance in practice has been discussed. However, the author is of the opinion that these drains will be, from the point of view of promoting crop or pasture production, somewhat inferior to the drains receiving other treatments.

Some further trials have been instituted in the hope that the effectiveness of the mole-tile drain can be made equivalent to that of the other treatments.

It is considered that two factors contribute to the inferior flows from the mole-tile drain. The first is the fact that the tiles may have become very closely abutted in the process of forcing them into the mole channel thus limiting the ready entry of water into the drain. This aspect is the subject of further investigation.

A second and probably more important factor is that the permeability induced in the clay above the mole channel by the passage of the plough was not sufficient to allow free movement of water to the tiles. The mole-drain plough used was fitted with a standard blade which is ½ in. thick. It is known from mole drainage investigations that the use of a blade 1-in. thick greatly increases the "shattering" of the clay above the mole channel. This has been found to facilitate movement of water to drains but induces rapid collapse of the mole channel and for that reason is not recommended in mole drainage practice. Where tiles are introduced into the mole drain, the collapse of the channel is unimportant and the effect of using a thick blade would be worthy of investigation. The mole-tile type of drain does not seem to have been used in New Zealand, though it has been overseas, particularly in Europe where techniques have been perfected to draw the tiles into the channel directly behind the plough.

If satisfactory, it would have an application on country which has a suitable natural grade and on which the ground is free from minor irregularities. This is a necessary prerequisite, since the mole plough, as at present designed, follows the surface irregularities closely. Refinements could no doubt be fitted to the implement to permit a certain amount of grading as the plough proceeds.

The method has the advantage that the tiles could be laid rapidly and comparatively cheaply, although the work would be limited to that time of the year when moles could be pulled.

Conclusions

It should be emphasized that the report presented here is of an interim nature since the experiment has been in progress only 10 years.

Nevertheless it seems desirable to draw some tentative conclusions from the data available.

(1) Such differences in drain performance as have been observed between the drains installed in the conventional manner (Treatments A–D) are not likely to produce a measurable difference in the growth of crops or pastures. Therefore, it would appear unimportant, in practice, which method of backfilling is used.

It is possible, however, that some treatments may become relatively less efficient with the passage of time. For example, the soil may work down among the stones in Treatment C and restrict the movement of water to the drains.

(2) It is sometimes claimed that a layer of straw over the tiles prevents the movement of fine soil particles into the tile drain. Since no silting was observed in any treatment in this experiment the use of straw for this purpose seems unwarrantd under the particular soil conditions. In more silty soils and for drains laid on flatter grades, it may be an advantage. Investigations into this aspect are being carried out.

(3) The method of laying tiles behind a mole plough as carried out in this trial has not proved as effective as installing them in the conventional manner. Where conditions favour the technique, however, the method offers promise. It is probable that the efficiency of the method might be improved by the use of a thicker blade and by taking steps to ensure that the tiles do not become too closely abutted in the course of introducing them into the mole channel.

(4) The need for further work to elucidate some of the fundamental factors involved in the movement of water to drains is evident. This is shown by the variations in outflows which cannot at present be adequately accounted for.

ACKNOWLEDGEMENTS

The author wishes to thank Professor A. W. Hudson for his constant help and advice during the conduct of this experiment. He is also indebted to Mr. N. L. St. George for his assistance with the field work and analysis of outflow records.

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FERMENTATION OF GLYCEROL IN THE RUMEN OF SHEEP

By A. T. Johns, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publication, 24 August 1953)

Summary

In order to investigate the claim that glycerol is more effective than glucose as a cure for sleepy sickness in ewes, the products of fermentation of these two materials in the rumen of sheep have been compared. It appears that glycerol is likely to be superior therapeutically to glucose as the former is converted to propionic acid, an antiketogenic substance, while the latter gives rise to a mixture of acetic, propionic, and butyric acids, the last of which is ketogenic.

Introduction

A disease of ewes known as pregnancy toxaemia or sleepy sickness is common in sheep carrying twin lambs or one large lamb. This is most prevalent when animals in good condition are subjected to serious under-nutrition during the month prior to parturition. Well known features of sleepy sickness are hypoglycaemia and an increase in ketone bodies, which indicate that glycogen stores of the liver are being exhausted and that fat reserves are being mobilised. The rational treatment would consist in feeding compounds which raise both the level of glycogen in the liver, and of sugar in the blood.

An apparent anomaly has been that while parental administration of glucose gives temporary improvement, oral administration of molasses or glucose has been of very doubtful value. In fact, Griffiths (1952) states that treatment is usually considered useless.

The work of Phillipson and Elsden (1948) established that the greater part of the energy requirements of the sheep are met by lower fatty acids which arise in the rumen by microbial dissimilation of carbohydrates. It is therefore unlikely that much of the orally administered glucose will be available as such for absorption into the blood stream of the sheep.

Griffiths (1952) decided to try glycerol as a treatment for sleepy sickness because glycerol can enter into the carbohydrate cycle and be converted into glycogen and glucose and so is antiketogenic. Both Griffiths (1952) in England and Malone (1952) in New Zealand have reported that glycerol drenching is remarkably effective in the treatment of sleepy sickness in ewes. Earlier, Johnson (1951) in America had demonstrated that the condition of ketosis in dairy cattle responds well to drenching with glycerol. No work has been reported on the fate of glycerol in the rumen to indicate whether it is absorbed unchanged into the blood stream or fermented by the rumen microflora.

The present paper presents results on the fermentation products of glycerol and makes a comparison with the dissimilation of glucose by the rumen microflora.

EXPERIMENTAL PROCEDURE

A sheep fitted with a permanent rumen fistula by the method of Jarrett (1948) was used as the source of rumen contents for *in vitro* fermentations and for the study of the fate of glycerol and glucose *in vivo*. The animal was on winter pasture (short rotation ryegrass-white clover) but had no access to food for 16 hr. before an experiment.

For *in vivo* experiments a sample of rumen contents was taken 1 hr. before adding the glycerol or glucose, and also immediately before the particular substrate was added through the fistula. The dose was 180 g. in 200 ml. water (approximate therapeutic dose) brought to 38° C. Samples of rumen contents were then taken at approximately hourly intervals.

In vitro fermentations were carried out at 38° C in 250 ml. conical flasks fitted with inlet and outlet tubes to permit gassing with carbon dioxide. The glucose or glycerol (2 g.) was dissolved in 25 ml. of $M/_{15}$ phosphate buffer pH 6.8 and the volume made up to 200 ml. with rumen contents that had been strained through muslin. A blank fermentation was run with the same amount of buffer and rumen contents.

Methods of analysis

Aliquots of 5 ml. were taken in both *in vivo* and *in vitro* experiments for analysis of glucose or glycerol, and cadmium hydroxide used as the clearing agent (Doak, 1939).

Glucose was analysed by the ferricyanide method of Fujita and Iwatake (1932).

Glycerol was determined by oxidation with periodic acid followed by measurement of the excess periodate (Neish, 1950a).

Initially, glycerol was separated from other rumen constituents by partition chromatography on wet celite (Neish, 1950b). This was later found to be unnecessary as other compounds oxidised by periodate were present in such low concentrations compared with the amount of glycerol as to cause no appreciable error in the determination.

Volatile acids

Total volatile fatty acids were determined by adding to 5 ml. rumen contents, 1 ml. of 10N H_2SO_4 and 4 g. $MgSO_4.7H_2O$ followed by steam distillation in the Markham still. Quantitative recovery of volatile acid was obtained by collecting 50 ml. of distillate. A further 50 ml. was collected as a blank. The distillate was titrated with 0.1 N NaOH under CO_2 free conditions.

Individual acids were determined by the gas liquid chromatographic method of James and Martin (1952) run at 100° C with the automatic titration unit. Butyric acid figures are the sum of the *n*- and *iso*-butyric fractions. Valeric acids have not been plotted.

RESULTS

In vivo

The results of adding (a) glucose, and (b) glycerol to the rumen of sheep are shown in Fig. 1. Glucose was fermented very rapidly, about 80 per cent disappearing in 30 min. with a consequent rise in

volatile acids. It is most probable that a rapid rise in lactic acid took place as an intermediate step between the glucose breakdown and fatty acid production (Elsden, 1945). Lactic acid was not estimated in these experiments.

Glycerol, by contrast, was fermented slowly with almost 50 per cent still present in the rumen after 7 hr. Consequently, volatile acid production was much more gradual than with glucose.

Fig. 2 illustrates the change in concentration of individual acids of the volatile acid fractions. With glucose as substrate, a rapid rise occurred in the amounts of acetate, propionate, and a slight rise in butyrate. The amount of propionate had started to drop rapidly after 3 hr.

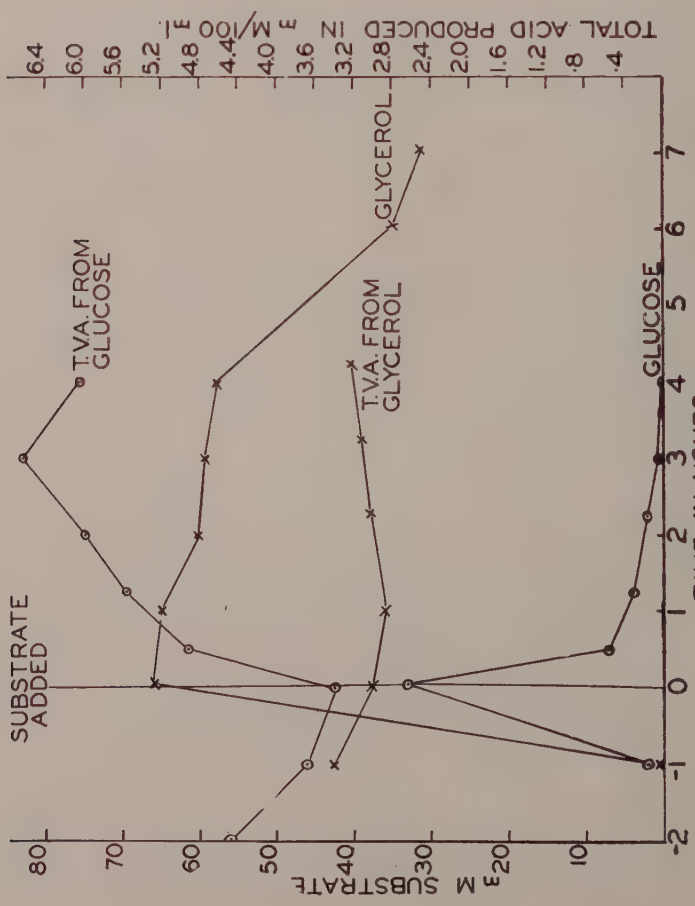

FIG. 1.—Conversion of glycerol and glucose into volatile fatty acids in the rumen of sheep. Substrate 180 g. in 200 ml. water.

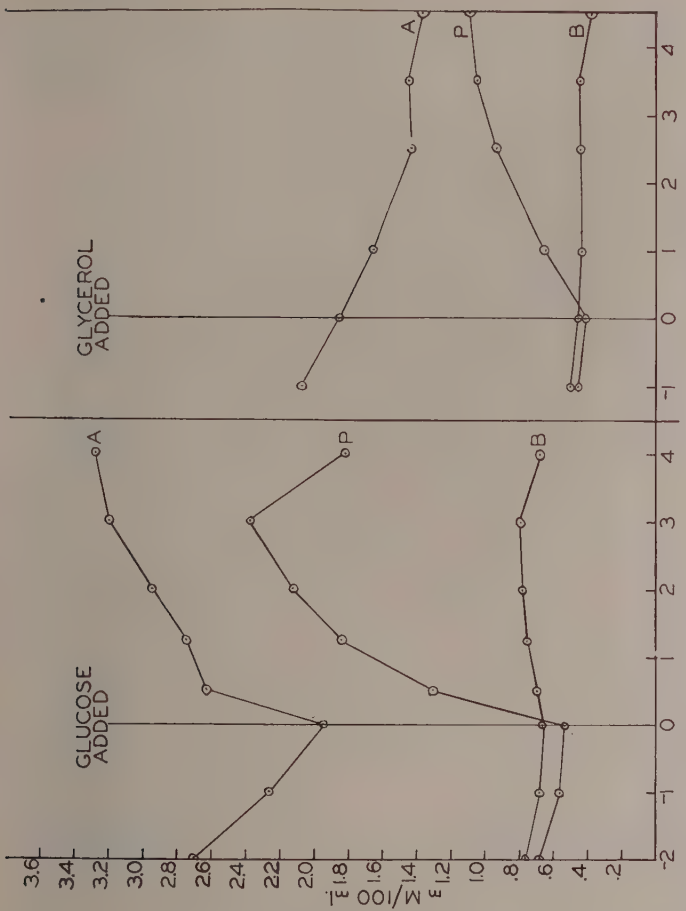

FIG. 2.—Constituent acids of the T. V. A. shown in Fig. 1. A. Acetic acid. P. Propionic acid. B. Butyric acid.

By contrast, fermentation of glycerol resulted in a slow rise in the concentration of propionate with a decrease in the amount of acetate, and little change in the concentration of butyrate. This indicated that the glycerol was being converted almost entirely into propionate.

In vitro

The products of fermentation, as determined from the rumen samples, are not a true measure of the amount of acid produced, as the acids in the rumen contents are: (1) diluted by the inflow of saliva; (2) passed on down the alimentary tract; (3) absorbed through the rumen wall into the blood stream; (4) metabolised by the rumen epi-

thelium, particularly butyrate (Pennington, 1952). In order to obtain a more accurate picture of the amounts of each acid produced, the *in vitro* fermentation of glucose and glycerol were carried out and the results given in Figs. 3 and 4. In the fermentation of glucose there was a considerable production of acetate while the production of both propionate and butyrate was approximately equal at a lower level than acetate. All the glucose had disappeared in 4 hr. and further volatile acid production would be from the lactate formed as an intermediate.

Glycerol gave rise almost entirely to propionate. The production of acetate and butyrate did not exceed that produced in the blank. After 8 hr., 50 per cent of the glycerol was still present. At 48 hr., the amount of propionate far exceeded the amount of othe other acids.

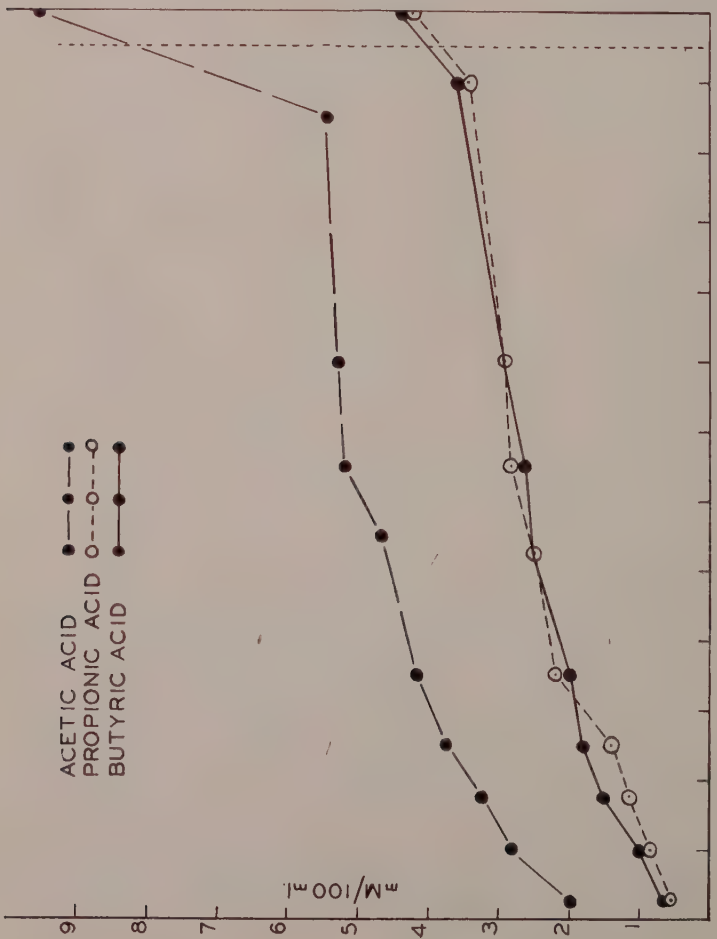

FIG. 3.—The *in vitro* fermentation of glucose. 2 g. glucose in 25 ml. M/15 phosphate buffer pH 6.8 + 175 ml. rumen contents gassed with CO_2. Blank values are the same as Fig. 4.

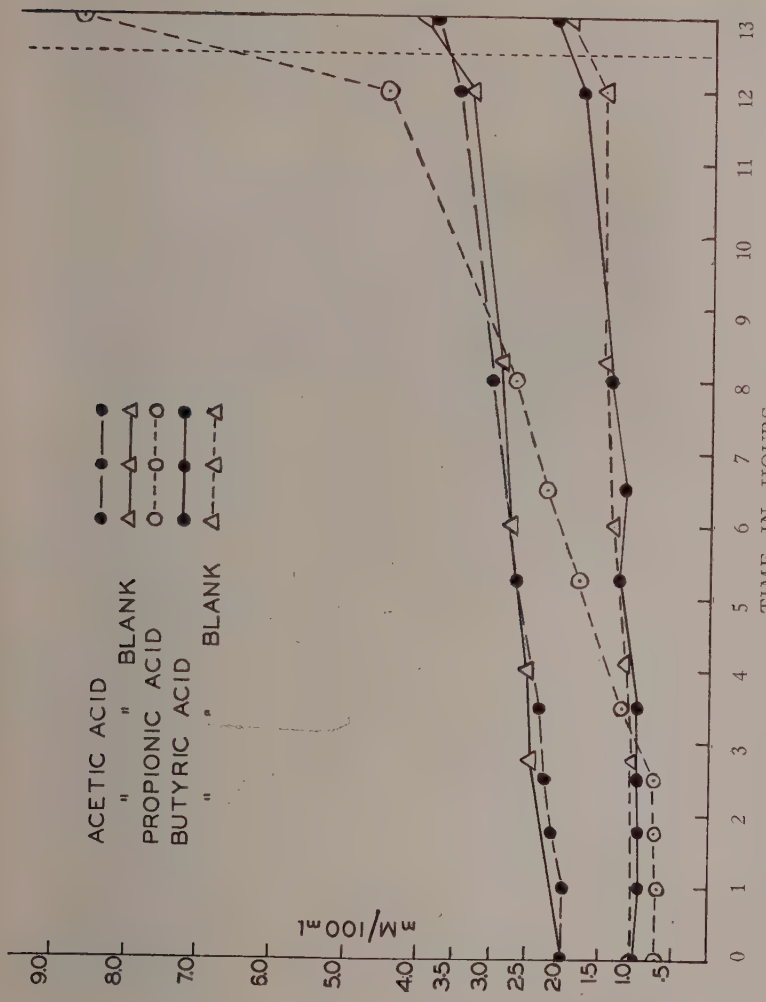

FIG. 4.—The *in vitro* fermentation of glycerol. 2 g. glycerol in 25 ml. M/15 phosphate buffer pH 6.8 + 175 ml. rumen contents. Acetic and butyric figures for blanks without glycerol plotted for comparison. Propionate blank level showed very little change from initial value.

DISCUSSION

In his study of the *Propionibacteria,* van Niel (1928) showed that glycerol fermented in yeast water gave rise to an almost quantitative yield of propionic acid. Glucose, on the other hand, was converted to acetic and propionic acids. Though the *Propionibacteria* have not been shown to be functional in the rumen (Johns, 1951), the results presented are what would be expected from this type of anaerobic fermentation. In the butyric acid type of fermentation (e.g., *Cl. butyricum*), a mixture of acetic and butyric acids are formed from glucose.

It will be noted that whereas glucose is fermented immediately *in vivo* and *in vitro* there is a lag period before production of propionic acid occurs from glycerol. This is particularly noticeable in Fig. 4 where little propionate is produced for 2¼ hr., and thereafter a steady rise in level occurs. This fermentation is slower than in the rumen, probably due to lack of stirring and accumulation of acids in the flask. The shape of the glycerol fermentation curve indicates either: (a) the formation of adaptive enzymes by the bacteria capable of fermenting glycerol (Karstrom 1930); or (b) an increase in the population of organisms present in the rumen capable of fermenting glycerol.

Traces of ketone bodies occur in the blood of normal animals, but the amounts present may be greatly increased when there is a large amount of fat metabolised. The energy requirements of an animal are normally met by metabolising fat and carbohydrate together, but if for any reason carbohydrate metabolism is subnormal, more fat is metabolised. In starvation, ketone bodies accumulate once the glycogen reserves of the liver have been exhausted.

Of the three fatty acids absorbed from the rumen only propionate is known to be glucogenic (Ringer, 1912). Jarrett *et al.* (1952) have shown that both acetate and butyrate when injected separately into sheep intraveneously were markedly ketogenic, whereas propionate caused a reduction in circulating ketone bodies. When equal proportions of acetate and propionate were injected, the presence of propionate prevented the rise in ketone bodies that followed acetate alone. Pennington (1952) has demonstrated that rumen epithelium can metabolise the lower fatty acids, 65-70 per cent of the butyrate metabolised being converted to ketone bodies.

Shultz and Smith (1951), as a result of studies on the administration of the three acids to normal ruminants by rumen puncture, obtained similar results to Jarrett *et al.* (1952) and suggested that, from the standpoint of ketosis, abnormally high production of propionic acid in the rumen would be desirable and excessive production of butyric acid would be undesirable. Shultz (1952) has employed sodium propionate successfully in the treatment of ketosis in dairy cows.

It appears probable that glycerol will be of importance in the alleviation of conditions of ketosis in ruminants for the following reasons:

(1) Part of the glycerol will be absorbed unchanged if not through the rumen wall then lower down the alimentary tract. Glycerol is antiketogenic by virtue of its being a precursor of liver glycogen. Teng *et al.* (1953) have shown with liver slices from fasting rats that, of the glycerol metabolised, 70 per cent was accounted for as glycogen and glucose, whereas only 23 per cent of the pyruvate metabolised was accounted for in that way.

(2) Glycerol is fermented slowly in the rumen to propionate, an antiketogenic substance available for absorption through the rumen wall into the blood stream.

Glucose, on the other hand, is very rapidly fermented in the rumen and very little will be available for absorption. Of the mixture of acids produced two may be ketogenic.

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DIE-BACK CONDITION OF PHORMIUM SEEDLINGS USED IN YELLOW-LEAF INVESTIGATIONS

By R. A. Cumber, Entomological Research Station, Department of Scientific and Industrial Research, Foxton

(Received for publication, 20 July 1953)

Summary

A sudden die-back of *Phormium* seedlings which appears to be distinct from *Oliarus*-induced yellow-leaf and may be due to fungal attack, is described.

Introduction

Recent advances in the study of the yellow-leaf condition of *Phormium* have been described in a series of papers by Boyce and Newhook[1], Strzemienski[2], Newhook[3], Cumber[4], and Boyce *et al.*[5], published in 1953. In describing the experimental work which indicated that the yellow-leaf condition could be transmitted by *Oliarus atkinsoni* Myers (Hemiptera, Cixiidae), the writer referred to several cases of deaths in both test and control plants "from causes undetermined".

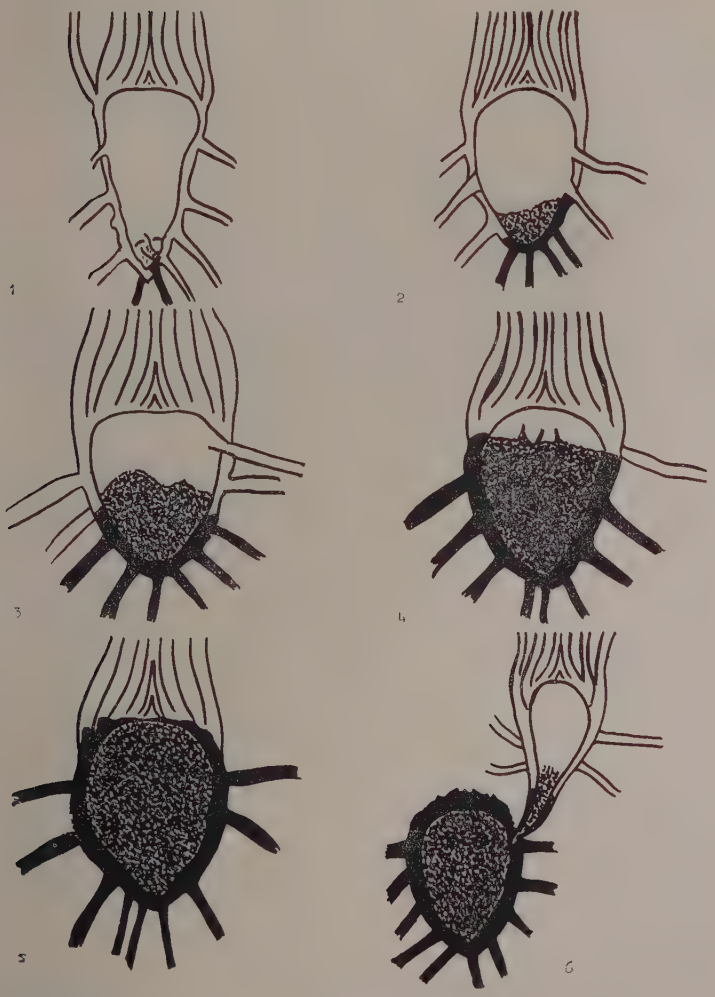
In further experiments to determine the virus-vector relationships of *O. atkinsoni*, and the importance of other possible vector species, a large number of plants have suffered from a sudden die-back with symptoms apparently distinct from those of *Oliarus*-induced yellow-leaf. Control and test plants were affected in approximately equal numbers. The condition was pronounced in seed boxes and pots, and to a lesser degree in plants growing directly in the ground. This sudden die-back was probably responsible for the anomalous deaths in the original work referred to above.

A description of sudden die-back is given below, and those symptoms of *Oliarus*-induced yellow-leaf which appear important in distinguishing the two conditions are detailed. The sudden die-back symptoms are those seen in experimental work analysed in June. Season and *Phormium* variety may affect symptoms to some degree.

Symptoms

Sudden Die-Back

The condition first appears as a small patch of dead tissue which is usually situated in the distal portion of the rhizome and may be initiated by diseased bundles coming from an adjacent dead main root (Fig. 1.). This small area is sometimes preceded by red vascular bundles which extend a few millimetres into the apparently healthy rhizome. The area of affected tissue advances up the rhizome, the deterioration in the parenchyma tending to keep pace or, in the severe condition, advancing ahead of any obvious reddening of the vascular bundles. Main roots are by-passed and die back (Figs. 2–5). The disease may advance more rapidly up one side of the rhizome, leaving living roots on the other side. In front of the advancing rotted area, the vascular bundles show no necrosis or gummosis with phloroglucinol and acid staining. In some cases where vegetative reproduction has produced new fans, the rot may be seen invading the connection with the parent plant (Fig. 6).



FIGS. 1-6.—Longitudinal sections through rhizomes of seedlings (2 years old, variety "301") to show stages in sudden die-back. Dead tissue is black or stippled.

The first symptom visible above ground may be a slight reduction in the size of the younger blades, the leaves being soft and not yellowed; or it may be a yellowing of the tips of one or several of the outermost blades. Occasionally there is a yellowing of one of the middle-aged leaves. By this time, considerable deterioration in the older portion of the rhizome has taken place. Marked symptoms above ground indicate that only a small portion of the rhizome is still living, the remainder being dark brown and hard, and sometimes containing small cavities.

As the rot reaches the apex of the rhizome the remaining roots die, and the leaves that are left suddenly roll throughout their length, the youngest ones still being green, the others yellow or reddish. Although it is possible that there is recovery from incipient stages, once this roll appears there is no recovery of the plant. A parent plant is often survived by the smaller fans which are disengaged and do not appear to be affected.

Oliarus-Induced Yellow-leaf

In experimental *Oliarus*-induced yellow-leaf the symptoms above ground are usually evident for a long period before the death of the plant. The rhizome shows widespread blockage from an early stage which is followed by general root collapse and the production of a few new roots. At the same time several of the outer leaves show some yellowing distally, and there is a reduction in the size of the younger leaves. Following this, there may be a seasonal period of attempted recovery, but the plant remains small and is generally yellowish, from which time it dwindles producing smaller and smaller leaves until it finally dies.

During this period, the distal portion of the rhizome darkens and becomes invaded by secondary organisms which soon destroy it. The zone of dead tissue advances towards the apex of the rhizome through a wide zone of translucent tissue containing mainly blocked, and sometimes reddish, vascular bundles.

Separation of the Two Conditions

In a comparison of symptoms produced by *Oliarus*-induced yellow-leaf and by sudden die-back, the latter appears to be characterized by: (1) absence of translucent tissue with blocked vascular bundles in advance of the rot zone in the rhizome; (2) the black (as opposed to brownish) colour and greater persistence of dead roots; (3) harder nature of the dead rhizome which may contain cavities; (4) sudden death with less obvious dwindling in size of leaves; and (5) rolling of leaves throughout their entire length while still green.

In *Oliarus*-induced yellow-leaf the symptoms are systemic and consistent with those of a virus infection, whereas in sudden die-back they appear to be regional and advance along a definite front which is more typical of fungal attack. In the field there may be a combination of these two conditions. The typical sudden roll of the die-back condition is often seen in full-grown plants in areas where typical *Oliarus*-induced yellow-leaf is also present.

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PLANT QUARANTINE

II. LIST OF PLANT DISEASES AND PESTS INTERCEPTED IN NEW ZEALAND IN 1952

By H. Jacks, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 20 July 1953)

Introduction

In this list are recorded plant diseases and pests intercepted in 1952 on plants and plant products introduced into New Zealand. Specimens were submitted by Horticultural Inspectors of the Department of Agriculture and identified by specialist officers of the Plant Diseases Division. Specimens submitted but found to carry organisms which could not be identified for various reasons, e.g., because of damage, failure in culture or rearing tests, etc., are not listed. Diseases and pests not established in New Zealand are marked with an asterisk.

LIST OF PLANT DISEASES AND PESTS INTERCEPTED IN NEW ZEALAND IN 1952

Disease or Pest		No. of Consignments	Host		Country of Origin	Discovered in:
Common Name	Generic Name		Common Name	Generic Name		
Diseases:						
Common-scab	*Actinomyces scabies* (Thaxt.) Gussow	1	Potato	*Solanum tuberosum* L.	Scotland	Napier
Coral-spot	*Nectria cinnabarina* (Tode) Fr.	1	Apple stocks	*Pyrus malus* L.	Holland	Auckland
Die-back	*Diaporthe perniciosa* Marchal	1	" "	" "	"	"
Dry-rot	*Fusarium* spp.	1	Onion	*Allium cepa* L.	Australia	Blenheim
Grey-mould	*Botrytis cinerea* Pers.	1	" "	" "	"	"
Leaf-spot	*Gloeosporium* spp.*	1	Orchids	*Orchidaceae* (?)	"	Auckland
Soft-rot	*Erwinia carotovora* (Jones)	1	Onion	*Allium cepa* L.	"	Blenheim
Spur-blight	*Didymella applanata* (Niessl.) Sacc.	1	Black currant	*Ribes nigrum* L.	Canada	Wellington
Stem-canker	*Fusarium latiritium* Nees.	1	Apple stocks	*Pyrus malus* L.	Holland	Auckland
Virus	Cucumber Mosaic	1	Daphne	*Daphne odora* Thunb.	Australia	Christchurch
Pests:						
Ants	*Pheidole megacephala* Fab.	1	Banana	*Musa paradisiaca* L. var. *sapientum* Kuntze	Fiji	Invercargill
Ants	*Camponotus* sp.*	1	Packing case	—	Ceylon	Wellington
Beetles	*Buprestidae* sp.	1	Black currant	*Ribes nigrum* L.	Canada	"
"	*Aphodius fimetarius* L.*	1	Mountain Laurel	*Kalmia latifolia* L.	Holland	Ashburton
"	*Necrobia rufipes* Deg.	1	Nutmeg	*Myristica fragrans* Houtt.	Samoa	Auckland
"	*Carpophylus hemipterus* L.	1	Dried fruit	—	Australia	Christchurch
"	*Anthicus* sp.	1	Straw	—	England	"
"	*Bruchidae* sp.	1	Croton leaves	*Codiaeum*, Juss. (?)	Fiji	Auckland
"	*Ramosia tipuliformis* (Cler.)	1	Black currant	*Ribes nigrum* L.	Canada	Wellington
Clear winged moth	*Plodia interpunctella* (Hub.)	1	Almond nuts	*Prunus communis* Fritsch	Italy	Christchurch
Cosmopolitan moth	*Thermobia domestica* (Pack.)	1	Cashew nuts	*Anacardium occidentale* L.	India	"
Firebrat (Silverfish)	*Hippelates* sp. (Fam. *Chloropidae*)	1	Aircraft	—	Fiji	Auckland
Flies	*Hylemia urbana* Mall.	1	"	—	Australia	"
"	*Drosophila melanogaster* Meig.	1	"	—	"	Christchurch
"	*Ortalidae* sp.*	1	"	—	"	"

Grubs	*Lassioderma serricorne* (Fab.)	1	Ginger	*Zingiber officinale* Roscoe	Jamaica	Dunedin
Hymenopteron fly	*Hymenoptera* sp.	1	Broad beans	*Vicia faba* L.	Burma	Wellington
Ichneumon fly	" "	1	Orange, sweet	*Citrus sinensis* Osb.	Jamaica	Auckland
" "	*Diadromus* sp.*	1	Aircraft	-	Australia	Christchurch
Larvae	*Diptera* spp.	1	Onion bulbs	*Allium cepa* L.	"	Blenheim
Longhorned beetle	*Captocercus-truncatus* Aur.	1	Timber	—	"	Timaru
Mealybugs	*Pseudococcus* sp.	1	Cactus	*Cactaceae* (?)	Mexico	"
Mediterranean Flour moth	*Ephestia kuehniella* Zell.	1	Dried fruit	—	China	Christchurch
Midge	*Chironomus* sp.	1	Aircraft	—	Australia	"
Mirid bugs	*Sixconotus* sp. and *Halticotoma* sp.	1	Cactus	*Cactaceae* (?)	Mexico	Timaru
Saw toothed grain beetle	*Oryzaephilus surinamensis* L.	1	Lotus nuts	*Nelumbium* sp. (?)	Hongkong	Dunedin
Mites	*Enemothrombium* sp. (Fam. *Leeuwenhoekidae*)	1	Mixed shrubs	—	Holland	Auckland
"	*Carpoglyphus lactis* L.*	1	Dried figs	*Ficus carica* L.	Turkey	Christchurch
"	*Pergamasus* sp. (Fam. *Parasitidae*)	1	Rhododendrons	*Rhododendron* L. (?)	England	Wellington
Moth	*Plodia interpunctella* Hubn.	1	Lotus nuts	*Nelumbium* sp. (?)	Hongkong	Dunedin
Mycetotophilid fly	*Sciaria agraria*	1	Orchids	*Orchidaceae* (?)	Australia	Wellington
Rove beetle	*Staphylinidae* sp.	1	Brazil nuts	*Bertholletia excelsa*, Humb. & Bonpl.	Brazil	Dunedin
Scales	*Hemiberlesia palmae* Cock.*	1	Pomelo grapefruit	*Citrus maxima* (Burm.) Merrill	Malaya	Wellington
"	*Lepidosaphes beckii* Newn.*	1	Lemons	*Citrus limonia* Osb.	Italy	"
"	*Aspidiotus hederae* Vall.	1	"	" "	"	"
"	*Lepidosaphes machili* (Mask.)*	1	Orchids	*Orchidaceae* (?)	Australia	Christchurch
"	*Lepidosaphes beckii* Newn.*	1	Orange, sweet	*Citrus sinensis* Osb.	Norfolk Is.	"
"	*Saissetia* sp.	1	Cactus	*Cactaceae* (?)	Mexico	Timaru
Termites	*Porotermes adamsoni*	1	Timber	—	"	N. Plymouth
Weevils	*Spermophagus subfasciatus* Boh.*	1	Broad beans	*Vicia faba* L.	Burma	Wellington
"	*Gerstaeckeria* sp.	1	Cactus	*Cactaceae* (?)	U.S.A.	"
"	*Calandra oryzae* L.	1	Lotus nuts	*Helumbium* sp. (?)	Hongkong	Dunedin
Worms	*Enchytraeidae* sp.	1	Orchids	*Orchidaceae* (?)	Australia	Wellington

BOOK REVIEW

THE INJURIOUS INSECTS OF THE BRITISH COMMONWEALTH

By J. W. Evans, M.A., D.Sc. Commonwealth Institute of Entomology, London. 242 pp.

The work covers the Commonwealth, with the exception of the British Isles, India and Pakistan, because (as the author points out) few exotic pests occur there, the agriculture systems being ancient ones; on the other hand, agricultural development in the other countries is recent and introduced pests are abundant.

Naturally, within the confifines of its 242 pages, all injurious insects cannot be dealt with; parasites and predators in biological control are referred to only when incidental to a special problem. The original plan was for a comprehensive work, but this had to be abandoned due to circumstances; one is aware of the author's disappointment in being unable to present the undertaking as he would have wished.

Nevertheless, in its abbreviated form, this work is a very useful one, especially in that it gives a rapid lead into regional problems, the references to relevant data in the Review of Applied Entomology further opening the way to securing more detailed data. Also in the section dealing with territories, useful data are presented on the climate, topography and products, and on the entomologists working in the areas.

The work is set out in five parts. The first part (37 pp.) deals with the principal insects in each Commonwealth Territory—Africa, Asia, Australia, Mediterranean Islands, New Zealand, North America and Bermuda, Pacific Islands, South and Central America and West Indies. The second part (21 pp.) is a crop host list, the country where each insect occurs being given. The third part (145 pp.) deals with the injurious insects according to orders, with information on each insect. The fourth part (7 pp.) is concerned with the biological control of noxious weeds in different countries. The fifth part (11 pp.) is on special problems and deals with plant quarantine, virus vectors, biological control of insects and research needs. There is an index of 21 pp.

—D.M.

PUBLICATIONS RECEIVED

1. Science and Fruit. Ed. by T. Wallace and R. W. Marsh. Commemorating the Jubilee of the Long Ashton Research Station, 1903-1953. University of Bristol. 1935. 308 pp. 30s.

This publication includes a detailed introduction surveying the development of the Station during the fifty years of its existence. The work is summarised under the following headings: Cider and Fruit Juices; Fruit Culture; Plant Nutrition; Plant Pathology; and Domestic Food Preservation. There are 30 pages of well-chosen illustrations.

This book concludes with a list of the staff since the foundation of the station.

2. The Journal of Soil Science. Ed. by G. V. Jacks. Vol. 4 No. 1, January, 1953. Oxford, Clarendon Press, 106 pp. 17s.

The first number of Volume 4 of this publication maintains the high standard set by the earlier issues. It includes, amongst others, papers on the following topics: Soil Science and Semantics; A Study in Podzolization examining the mobilization of Iron and Aluminium by Scots Pine Needles; Studies in Pedogenesis; and a Review of Literature relating to Nutrifying Bacteria.

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